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Report to the Local Government Board upon
the effects of certain condensing and drying
processes used in the preservation of
milk upon its bacterial contents, by Dr. S.
Delépine.

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REPORT

OF THE

UNITED STATES GOVERNMENT

FOR

THE YEAR 1900

AND

FOR THE YEAR 1901

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W. H. HARRIS, JR.



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Report to the Local Government Board upon the effects of certain condensing and drying processes used in the preservation of milk upon its bacterial contents, by Dr. S. Delépine.

ARTHUR NEWSHOLME,
Medical Officer,
December, 1914.

SCOPE OF THE INVESTIGATION.

Three methods of preservation of milk as practised at four factories situated in England were under investigation, viz.:—

- A. Manufacture of sweetened condensed milk. (Factory V.)
- B. Drying of milk over heated revolving cylinders. (Factories X and Y.)
- C. Drying by spraying the milk into a current of hot air. (Factory Z.)

The main object of the first set of experiments, conducted during the year 1910, was to ascertain whether *tuberculous cows' milk was still capable of conveying tuberculosis* after being treated according to methods A and B.

In the second set of experiments, carried out during the year 1911, special attention was paid to the general effects upon the bacterial contents of milk which followed the use of methods B and C.*

I. GENERAL OUTLINE OF EXPERIMENTS.

First set of experiments.—Tuberculous cows' milk was condensed at Factory V and dried at Factory X, where the methods A and B were practised on a commercial scale. This tuberculous milk was treated exactly as any other milk would have been treated. Samples of the milk were taken before, during and after treatment, great care being taken to guard against any accidental contamination.

* The reports upon these experiments were completed in 1911 and 1912, respectively, but their publication has been delayed pending the making of some intercurrent inquiries.

These samples were submitted to the following tests:—

- (1.) Estimation of solids and reactions, microscopical examination, &c.
- (2.) Cultivation on various media.
- (3.) Inoculation of guinea pigs.
- (4.) Feeding of rabbits.

Tuberculous milk.—Out of several cows with tuberculous udders, kept under observation for some weeks, only one was still secreting a fair amount of tuberculous milk when the time of the experiments arrived.

The amount of milk obtainable from that cow (1,340 c.c.) was not thought sufficient to infect the 60 gallons (272,000 c.c.) of milk needed for the tests, to such an extent and so uniformly that every drop of that experimental milk would contain tubercle bacilli easily demonstrable by direct microscopical examination. In order to obtain this result I found it necessary to add to the fluid cultures of a virulent tubercle bacillus (128) which I isolated in 1902 from the oesophageal gland of a tuberculous calf. In the course of the past ten years subcultures of this bacillus had been frequently tested by inoculation and feeding experiments, and invariably found virulent.* Cultures on glycerin potato, glycerin milk and plain milk incubated for 36 days at 37° C. were used for the purpose indicated above.

The following particulars are of importance not only for the purpose of estimating the infective power of the tuberculous milk, but also for that of showing how great an amount of fresh or prepared milk may be infected by a single cow. The cow in question was discovered on January 12th, 1910, in the course of inspection of a large herd, the mixed milk of which had been found in the previous month to be capable of infecting guinea pigs inoculated with it in my laboratory (M.B. 8,662).

On January 12th the left anterior quarter of the udder was clearly diseased. The sediment of 40 c.c. of milk from that quarter only was found capable of causing extensive tuberculous lesions in two guinea pigs, less than three weeks after inoculation (M.B. 8,747). The mixed milk of the three other quarters also produced tuberculosis, but in a slighter degree. Between the 20th January and the 15th February I examined *microscopically* the milk of each quarter separately on six different occasions with the following results:—

Quarters of the udder.	Quantity of milk secreted.	1st Exam.	2nd Exam.	3rd Exam.	4th Exam.	5th Exam.	6th Exam.
Left anterior	diminished	†††	†††	†††	†††	†††	†††
Right anterior	abundant	†	†	††	††	†††	†††
Left posterior	„	O	O	O	†	†	†
Right posterior	„	O	O	O	O	O	O

(Meaning of signs: O, no bacilli found after careful search; †, a few; ††, a moderate number; †††, many bacilli found.)

* The word “*virulent*” as used in this report is intended to convey the idea of *infectivity* as well as of *toxicity* and of other *pathogenic actions*.

On February 20th I controlled the microscopical findings by inoculation tests with the following results:—

Quarters of the udder from which milk taken.	Quantity of milk injected.	Effects of subcutaneous inoculation of guinea pigs (2134 A and B and 2135 A and B) in 3 weeks.
4 quarters' (mixed)	l.c.c.	Well marked tuberculosis.
Left posterior quarter	l.c.c.	"
Right anterior quarter	l.c.c.	"
Right posterior "	l.c.c.	No trace of tuberculous lesions.

This cow continued therefore to yield daily, for more than two months, highly infectious milk.*

On February 10th, although the amount yielded had gradually diminished, I was able to obtain the 1,340 c.c. of tuberculous milk used in the experiments.

Cultures.—The virulence of each culture of bovine tubercle bacilli (128) added to the experimental milk was tested separately. Some of these tests formed part of the control tests recorded in subsequent parts of this report.

In order to insure a thorough admixture of the natural tuberculous milk, the cultures and the fresh non-tuberculous milk I took the following precautions: The growth on each potato was removed carefully and mixed in a 250 c.c. flask with a small amount of sterilized milk and with a certain amount of milk cultures. The flasks, about $\frac{4}{5}$ th full, were shaken violently in a shaking machine for one hour. These separate emulsions were thrown into a larger vessel and shaken together for another hour. This highly infected milk prepared at the laboratory was then gradually mixed and thoroughly stirred with the amount of fresh milk needed for the experiment at the factories.

The proportions were as follows:—

The 50 gallons of milk (227,300 c.c.) used in experiment A contained the following amount of infective material:—

1,100 c.c. of fresh tuberculous milk from the cow (M.B. 8,747).

1,000 c.c. of milk cultures of the bovine tubercle bacillus (128).

15 potato cultures of the same bacillus (128).

The 10 gallons of milk treated in experiment B contained:—

240 c.c. of fresh tuberculous milk from the cow (M.B. 8,747).

250 c.c. of milk cultures of the bovine tubercle bacillus (128).

5 potato cultures of the same bacillus (128).

I ascertained by microscopical examination of the component parts that tubercle bacilli were evenly distributed, and that each

* Although these observations do not bear directly upon the subject of this report the continued absence of tubercle bacilli from the milk of one quarter while the milk of the three other quarters of the udder contained tubercle bacilli in increasing numbers, does not support the view held by some observers that tubercle bacilli are frequently secreted by a sound udder. In this case the three quarters secreting tubercle bacilli were the seats of gradually increasing tuberculous lesions, and the sound quarter secreted milk in which no tubercle bacilli could be found by me at any time during the course of a whole month.

drop of the final mixture actually used in each experiment contained tubercle bacilli easily demonstrable.

Portions of the same mixture were also tested by inoculation as will be seen in the records given in the report on each experiment. It will be sufficient here to state that the *number and virulence of the bacilli present in the treated milk were not in excess of what I had observed in the case of naturally infected milk, i.e., milk from cows with tuberculous udders, such as had been examined on more than one occasion in my laboratory.*

Second set of experiments.—For the purpose of ascertaining the effects which the two drying processes investigated had upon the general bacterial contents of milk, two factories (Y and Z) were visited, while at work, and samples were taken by us of the material treated, before, during, and after treatment, with all the care necessary to prevent any accidental contamination.

These samples were taken to the laboratory, where I examined them without delay with the object of finding:—

- (1.) The total amount of solids in each sample.
- (2.) The number of bacteria cultivable on various media, &c.
- (3.) The characters of the most important types of bacteria found in the finished product, and the relations of these bacteria to those present in the original milk.
- (4.) The thermo-lethal point of the most characteristic organisms.

Collateral investigation.—I thought it desirable to ascertain whether, apart from any infective property, preserved milk prepared with fresh milk containing a very large number of tubercle bacilli might have any toxic or other deleterious properties. Special attention was therefore paid to the condition of rabbits fed with various kinds of milk, fresh and preserved, infective and not infective.

II. OBSERVATIONS AND EXPERIMENTS RELATING TO METHOD A. (MANUFACTURE OF SWEETENED CONDENSED MILK.)

Short description of method.—On February 16th, 1910, Dr. Coutts and I visited Factory V, where the manufacture of sweetened condensed milk was conducted on a large scale, and under what appeared to us exceptionally good conditions.

The various operations to which the milk is submitted may be briefly described as follows. (See also Diagram 1.)

1. On arrival from various farms the milk is submitted to some preliminary tests, after which it is strained through fine copper gauze and weighed.
2. The strained milk accumulates in a large tank (capacity 1,000 gallons), whereby its composition is equalised.
3. (a) It is then heated in cylinders of a capacity of 200 gallons.

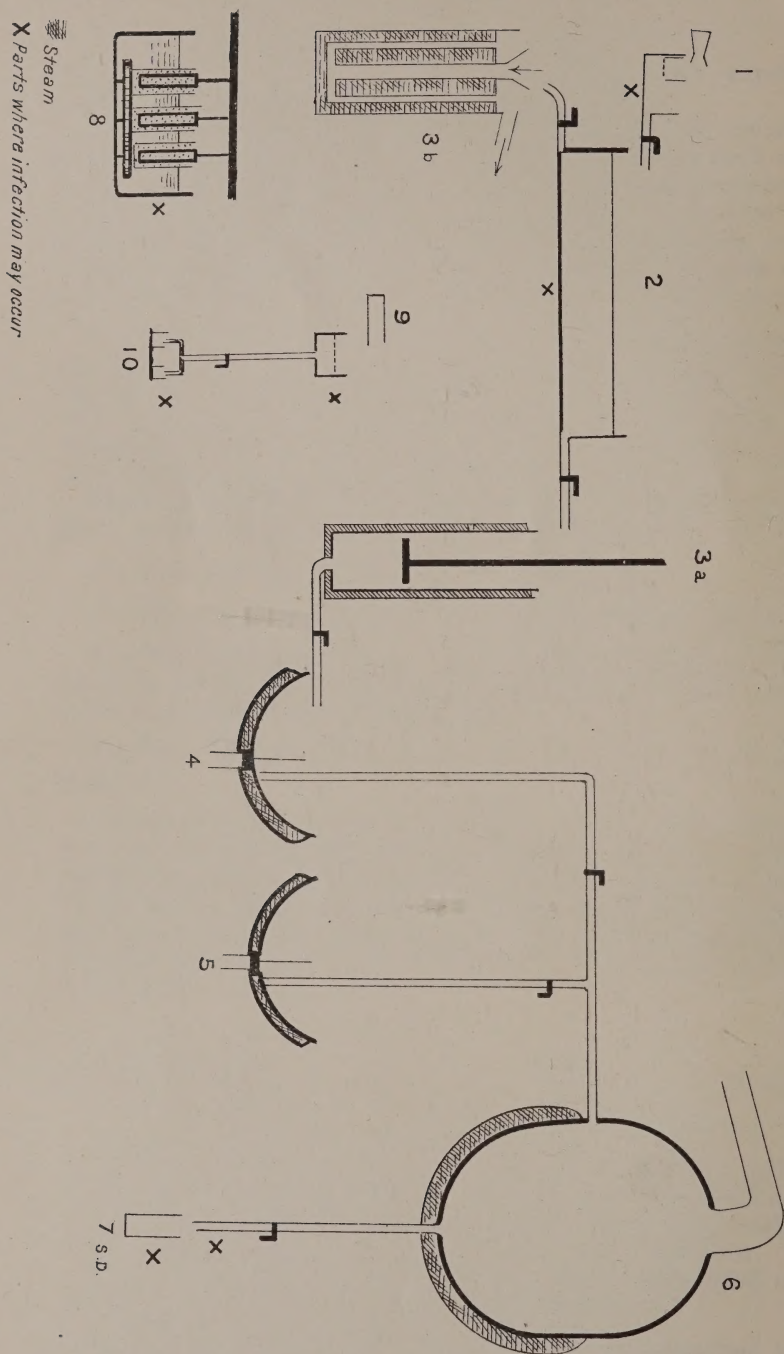


DIAGRAM I. Method A. The figures correspond to the stages described in the text.

Each cylinder is heated by steam admitted to the jacket surrounding it. The temperature of the milk can be raised from 75° to 80° C. in from 20 to 25 minutes. Whilst the milk is being heated it is continuously stirred by means of a vertical plunger. (This method of pasteurisation is not the only one used at the factory. Some of the milk is pasteurised by another method. See alternative method of pasteurisation, 3 (b), p. 7).

4. From the cylinders the milk is transferred to steam-jacketed pans, where it is heated in about six minutes to a temperature of 92° C.

5. This milk is mixed with a solution of cane sugar which has previously been boiled in another steam-jacketed pan. Whilst this is taking place the mixture is gradually transferred to the evaporating vacuum pan. Some of the pasteurised milk, not mixed with sugar, is generally transferred to the vacuum pan before the sugar mixture is admitted.

6. In the vacuum pan a vacuum equal to 65 to 70 centimetres, indicated by the manometer connected with the exhaust pipe, is maintained, and the milk is heated by means of the steam jacket and steam coil to a temperature of 40° to 45° C., at which the fluid passes into a state of ebullition. The evaporation is allowed to proceed until the milk is of proper viscosity, which occurs when the fluid has been reduced to about one-third of its original volume.

7. From the vacuum pan the condensed milk is made to run into cooling cylinders.

8. These cooling cylinders are transferred to water tanks in which they are made to rotate for two to three hours. During that time the milk is constantly stirred by means of stationary wooden stirrers.

9. The condensed milk is then passed through a copper gauze strainer.

10. The tins are filled automatically with the strained milk. The tins are new and clean, but not sterilized.

11. The tins are closed mechanically.

12. The soundness of the tins is tested.

Immediately after any part of the apparatus has been used and is empty again it is thoroughly cleaned and made ready for further use.

It is generally estimated that a hundred litres of milk yield about 31,500 grammes of condensed milk, or, roughly speaking, the finished product is about 31 per cent. of the original milk. During the preparation 11 to 15 per cent. of saccharose (cane or beet-root sugar) is added. The total amount of solids contained in the condensed milk should be about 74 to 79 per cent. of which about 57 per cent. is cane sugar.

Description of experiment. Nature of Samples.

Owing to the waste which would have been involved if the process had been conducted on the usual scale, and also to the

difficulty of infecting uniformly and thoroughly very large quantities of milk, we substituted for the large steam-jacketed cylinders one of the smallest steam-jacketed pans in which the milk was stirred by means of a wooden paddle instead of being stirred by a vertical plunger. Great attention, however, was paid to the temperature of the treated milk and to the duration of each stage of the process, and the results obtained are quite comparable with those which would have been obtained if the large cylinders had been used. With the exception of this slight departure the condensation of our infected milk was conducted on the usual lines.

The smallest amount of milk which could be condensed under these conditions was 50 gallons.

Three 17-gallon cans of fresh milk which had just arrived at the Condensery were supplied to us by the Company (*Sample 1* taken). This milk was infected in the way which has already been described (page 3).

The infected milk was poured into a cold steam-jacketed pan at 11.15 a.m. The temperature of the mixture when finished was 12° C. (*Sample 2* taken.)

11.16 a.m. Steam was turned on and the milk kept in constant rapid circular motion by a wooden paddle. A continuous record of changes of temperature was taken by means of a recording thermometer. The temperature was also taken at intervals with an ordinary thermometer with the following results:—

11.26 a.m.	Temperature	58° C.
11.31 a.m.	„	68° C.
11.32 a.m.	„	72° C.
11.34 a.m.	„	77° C. (<i>Sample 3</i> taken)
11.35 a.m.	„	78° C.

At 11.37 the steam was turned on more fully to raise the temperature:—

11.37 a.m.	Temperature	80° C.
11.41 a.m.	„	85° C.
11.43 a.m.	„	90° C.
11.45 a.m.	„	92° C. (<i>Sample 4</i> taken)

Steam stopped and pressure in jacket allowed to fall.

11.47 a.m. Temperature slightly under 93° C.

Meanwhile, 76 lbs. of beetroot sugar had been dissolved in about an equal amount of water and boiled in another steam-jacketed pan. The temperature of the boiling syrup reached 103° C. This sugar solution was afterwards transferred by means of metal cans to the pan containing the pasteurised milk, but not till after about 2/3rds of that milk had been transferred to the vacuum pan. Before being mixed with the milk the temperature of the syrup had fallen to about 94° C.

Whilst the previous operation was being conducted the vacuum pan was being “sterilized” by steam. The thermometer indicating the temperature inside the pan did not rise to more than 85° C. during that operation. The air was partially exhausted.

The following records will indicate the further course of operations and the temperatures and pressures indicated by the

thermometer and the vacuum gauge connected with the vacuum pan.*:—

		Readings on thermometer attached to pan.	Readings on vacuum gauge.
11.53 a.m.	Temperature in the vacuum pan ...	60° C.	—
12.0	Milk begins to reach the vacuum pan	58° C.	—
12.1 p.m.		45° C.	—
12.5		43° C.	70 c.m.
12.7		39° C.	69 c.m.
12.10		39.5° C.	68.5 c.m.
12.13		41° C.	68 c.m.
12.14		42° C.	68 c.m.
12.16		43° C.	67.5 c.m.
12.19		44° C.	67.5 c.m.
12.30	Mixture of milk and sugar now admitted	46.5° C.	67 c.m.
12.36		48° C.	66 c.m.
12.45		48° C.	67.5 c.m.
12.50		50° C.	66 c.m.
12.55	No more milk and sugar admitted ...	50° C.	67 c.m.
12.57	<i>Sample 7 taken by means of apparatus used for testing viscosity.</i>		
1.4		51° C.	65 c.m.
1.5		50° C.	69 c.m.
1.7		48° C.	68 c.m.
1.11	The operation was stopped. The viscosity was then slightly greater than desired, showing that the concentration had exceeded the usual limits.		
1.14 *	<i>Sample 8 taken as the condensed milk escaped from the vacuum pan into the cooling cylinder through the delivery pipe.</i>		
1.15	Cooling cylinder placed in cooling bath and rotated.		
2.35	<i>Sample 9 taken from rotating cooling cylinder when the milk was nearly cold.</i>		

Alternative Method of Pasteurisation. (Diagram 1, 3b).

Instead of being "pasteurised" in the steam-jacketed cylinders with plungers the milk is sometimes heated in another apparatus, which is composed of a series of steam-jacketed tubes through which the milk is made to pass at such a rate that before

* It will be noticed that the pressure oscillated between 65 and 70 c.m. and the temperature between 39° and 51° C. There was no proper correlation between pressure and temperature. This was probably due to the way in which the recording instruments were connected and also to the disturbance frequently caused by the admission of pasteurised milk to the vacuum pan.

it leaves the heater its temperature is raised to about 86°C . The filling of this heater was begun at 12 o'clock, the cold milk being admitted through a central tube going to the bottom of the cylinder and rising again through smaller tubes parallel to the central tube before passing out. In order to test the working of this apparatus I lowered the bulb of a recording thermometer into the central tube (through which the cold milk was admitted) to a depth of three feet (12.10 p.m.) The bulb of another recording thermometer was pushed to the same depth into one of the smaller peripheral tubes (through which the heated milk was rising). A quarter of an hour after the beginning of the experiment the bulb of the first thermometer was transferred from the central tube to the general outlet by which the heated milk left the apparatus. The temperature of the milk in the large tank feeding the heater was about 12°C . The temperature recorded by the thermometer in the central tube of the heater (about half-way down) remained practically the same as that of the milk in the tank. In the small peripheral pipe the temperature rose rapidly to 86°C . (12.15 p.m.), after which it oscillated between 74° and 88° , being generally above 80° . This oscillation of temperature was due to the variations in the amount of cold milk admitted to the heater by the operator whose duty it was to admit more milk when the temperature had a tendency to rise and less when it had a tendency to fall. His guide was an ordinary thermometer plunging in the milk running out of the heater at the general outlet. As I have previously said, the bulb of one of the recording thermometers was transferred about 12.25 p.m. from the central tube to the outlet; by means of this thermometer I ascertained that the temperature of the milk leaving the heater was much more even and slightly higher than that of the milk rising in the small peripheral tube at a depth of three feet from the surface. The temperature of the milk leaving the heater oscillated between 82°C . and 88°C ., being generally slightly above 86°C . (See Temperature Curves, Diagram 2.).

Samples of milk were taken at 12.15 p.m., after about 40 gallons of milk had already run through the heater, and the apparatus appeared to be in full working order.

Sample 5, from the large feeding tank. Temperature of the milk at the time 12° to 13°C .

Sample 6, from the outlet pipe of the heater. Temperature of the milk 86°C .

Some features of the samples.

There is nothing to be added to the previous description of the material employed for infecting the 50 gallons of milk used in the experiment. The infected milk appeared to be of average quality, and fairly clean. Its reaction was faintly acid to litmus.

The sediment of the samples taken from the mixtures containing sugar was more abundant and darker than the sediment of the original milk. The finished experimental condensed milk was thicker and obviously slightly more concentrated than average condensed milk of the same make. This was due to the fact that owing to the small amount of milk treated, the operator who

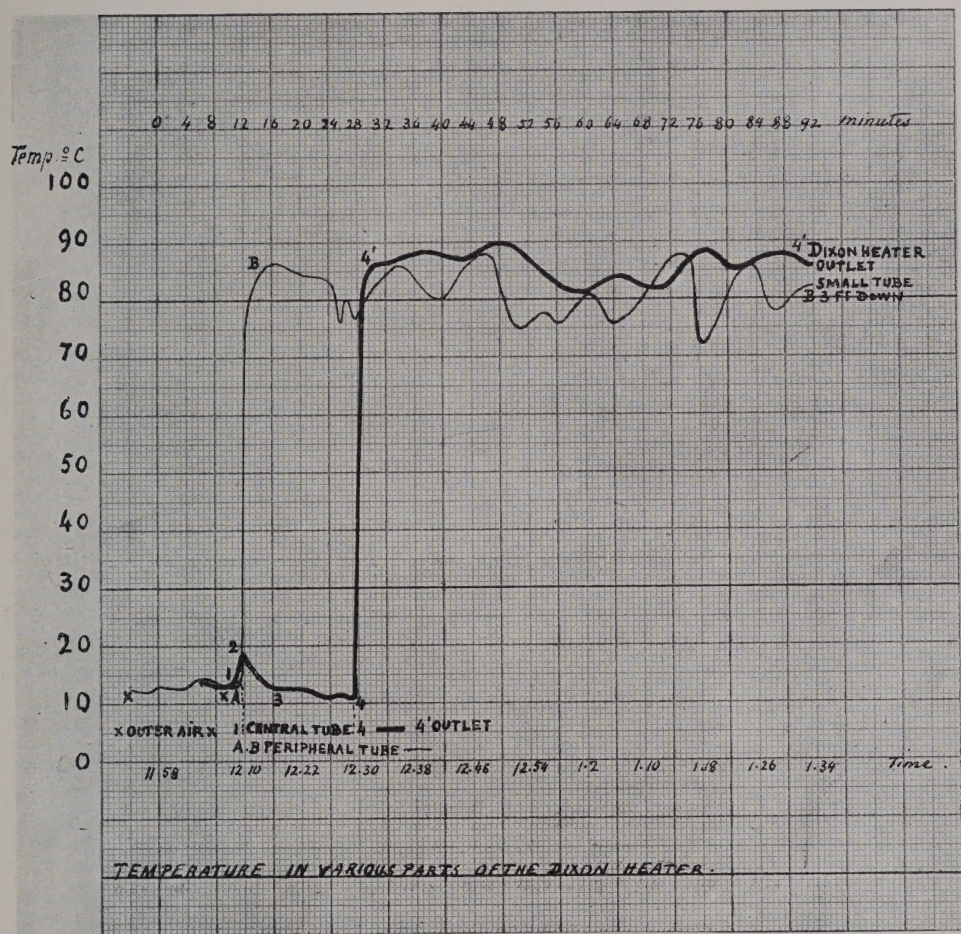


DIAGRAM 2. TEMPERATURE CURVES OF MILK DURING ITS PASSAGE THROUGH THE TUBULAR HEATER.

(Transferred accurately from actual records on curvilinear paper.)

tested the viscosity of the milk experienced greater difficulty than usual in ascertaining quickly enough the changes taking place towards the end of the operation.

The total amount of solids not volatile at 100° C. in some of the samples was estimated with the following results:—

Samples.		Total Solids. Per cent.	Reaction to litmus.
Sample 5. ...	Original milk before infection ...	12.384	Faintly acid.
„ 4. ...	Infected milk, heated to 92° C. ...	12.33	„ „
„ 7. ...	Infected milk, after addition of cane sugar	54.78	„ „
„ 9. ...	Infected milk, after fully condensed.	81.26	„ „

The proportion of solids in the finished product was slightly greater than usual; this agrees with the statement made by the operator to the effect that condensation had been pushed somewhat further than he desired.

Cultivation tests of the samples.

With regard to the average bacterial contents, the following observations were made:—

A.—1 c.c. of each sample mixed with 5 c.c. of sterilized separated milk, heated for 20 minutes at 80° C., incubated for 48 hours at 37° C. (aerobically) yielded in each case an abundant growth of motile sporing bacilli, mixed with a smaller number of cocci, producing within 48 hours partial coagulation and peptonisation, with very little, if any, smell.

B.—Similar mixtures treated anaerobically yielded also a growth of bacilli with production of a bulky gelatinous clot. A doubtful butyric smell was observed in some cases. In the cultures made with the original milk there was a distinct production of gas, but no gas production was observed in connection with the samples of treated milk.

C.—Plate cultures were made with dilutions corresponding to 1/100th, 1/10,000th, 1/100,000th and 1/1,000,000th of a cubic centimetre of the various samples; the water lost during the condensation was replaced in Samples 8, 9 and 10 so as to reconstitute the milk. In each case a set of peptone bouillon gelatine plates and a set of litmus lactose agar plates were made. The gelatine plates were incubated at 20° C. and the agar plates at 37° C. The colonies were counted on the third day and examined again on the fifth day. The numbers of colonies found on the third day are given in Table I.*

* N.B.—The largest amount of material used was 1/100th of one cubic centimetre in each case; when all the plates remained sterile the number of bacteria is given in the table as less than 100. It is obvious from the incubation experiments previously recorded that none of the samples were quite sterile.

METHOD A.—(MANUFACTURE OF SWEETENED CONDENSED FULL-CREAM MILK.)
TABLE I.—Summary of results of plate cultures tests.

No. of samples in text of report.	NATURE OF SAMPLES.	Gelatine plates incubated for 3 days at 20° C. No. of colonies per 1 c.c.	Litmus lactose agar plates incubated for 3 days at 38° C. No. of colonies per 1 c.c.
	<i>Mixed milk infected with tubercle bacilli—</i>		
2	50 gallons of milk before treatment (12° C.)	38,000,000	18,950
3	Same milk heated up to 78° C. (ordinary pasteurisation)	under 100	about 200
4	" " " 92° C.	under 100	under 100
7	" " " from vacuum pan, 2' after addition of sugar (temp. 50° C.)	under 100	about 200
8*	" " " from outlet of vacuum pan (reconstituted) (temp. 48° C.)	about 900	under 100
		(of which moulds 500)	
9*	2nd examination 112 days later	under 10	about 40
	" " " from cooling cylinder (reconstituted)	under 100	about 200
	2nd examination 112 days later	about 30	about 60
10	" " " from tins ready for sale (reconstituted)	under 100	about 300
	<i>Mixed milk not infected with tubercle bacilli—</i>		
5	From the 1,000 gallon tank of milk before treatment (12° C.)	24,000,000	17,400
6	After 40 gallons of milk have passed through heater (2nd method of pasteurisation) 86° C.	under 100	about 800

N.B.—Cultures made by adding 10¹⁰th of a gramme of condensed milk (samples 8 and 9) to peptone bouillon yielded in each case a growth containing bacilli and cocci, so that at least two kinds of bacteria were present in each 0.01 gramme of condensed milk. There must have been, therefore, at least 200 bacteria per gramme. It is obvious that some of the bacteria present in the condensed milk did not grow in three days on the solid media. From most of the agar plates prepared with the treated and untreated milk I was able to isolate sporing bacilli closely allied to or identical with the *B. mesentericus ruber* and the *B. mesentericus vulgaris*.

Summary of results of cultivation tests.

The results recorded in Table I. show that the mixed milk before treatment was rich in bacteria growing at ordinary temperature, but less so in bacteria growing at blood temperature. As was to be expected, the addition of tuberculous material did not materially increase the number of bacteria growing on gelatine or on agar at ordinary or at blood temperature. The effect of pasteurisation was to reduce to a very considerable extent the number of bacteria of all kinds, the reduction being more marked in the case of bacteria growing at ordinary temperature than in that of bacteria growing at blood temperature. No attempt was made to determine the nature of the bacteria present, as this was not the object of this part of the investigation. The cultivation tests were used simply to obtain a rough index of the original state of the milk and of the general effects of the treatment at its various stages. It will be noticed that the bacteria were at once reduced to a very small number after the first stage of pasteurisation at 77° C. A marked increase in the number of bacteria was noticeable in the case of Sample 8. This may be attributed to the presence of some bacteria and moulds in the delivery pipe used to empty the vacuum pan, the presence in one set of cultures of a large number of moulds is in favour of this supposition. Groups of tubercle bacilli were found in the finished condensed milk by direct microscopical examination, the bacilli were smaller and more granular than those found in the original infected milk. They looked shrunken.

Experimental and market samples of condensed milk compared.

The number of bacteria found in our experimental condensed milk was distinctly smaller than that found in a number of samples of condensed milk examined in my laboratory in previous years. Among these samples there were several of the same make as that investigated by us at the Factory V. The number of bacteria had seldom been found to fall below 1,000 per gramme. The number of bacteria in one sample of milk (prepared according to method A) reached 161,000 per gramme, in several other samples of the same kind of milk the number exceeded 20,000. I was therefore extremely surprised to find that the samples collected by Dr. Coutts and myself usually contained less than 100 bacteria per gramme. Although bacteria may remain alive for a considerable time in sweetened condensed milk they do not as a rule multiply in it, but in view of the differences mentioned above I thought it best to estimate again the number of bacteria contained in our experimental samples after allowing nearly four months to elapse after the preparation of the milk. The plates made on June 16th yielded nearly the same results as the plates made on February 24th.

Cultures on gelatine and on agar do not reveal all the bacteria present in the milk. This is proved by the fact that when a quantity of condensed milk, say, 1/100 gramme, which generally

yielded no growth when cultivated on nutrient gelatine, was added to bouillon and incubated at 37° C. a fairly abundant growth of two or three different kinds of bacteria (cocci and bacilli) usually appeared within 24 hours.

I think that the explanation of the difference in the bacterial contents of the samples collected by us at Factory V. and of the various samples purchased in the market is that at Factory V. condensed milk was more carefully prepared than at some other places.

This explanation seems all the more probable because the condensery, where our experiments were conducted, was extremely well equipped and appeared to be exceptionally well managed. Great cleanliness prevailed everywhere, and all the operations were conducted very systematically.

Opportunities for contamination during manufacture of condensed milk.

Very great care is needed during the latter stages of preparation to prevent contamination of the nearly sterile product. There are opportunities for contamination when:—

- (a) The milk is drawn from the vacuum pan into the cooling cylinders.
- (b) The cooling cylinders are rotated and the milk stirred by wooden stirrers.
- (c) The cold condensed milk is strained.
- (d) The tins are filled with the finished cold milk.

Previous to reaching these stages the milk is partly sterilized, and the number of bacteria is considerably reduced, but after this no further sterilization takes place, and the milk comes in contact with various articles which are not sterilized, and the purity of which depends on the cleanliness of the operators and on the sterility of the water and of the appliances used.

While the milk is in the cooling cylinders it may readily be infected either by the unsterilized cylinder, or by the unsterilized wooden paddles used to stir it, or by the water contained in the tank in which the cylinders are rotated for nearly three hours. The tins used for the final canning are new tins, but they may have been exposed to dust. The samples collected by us did not give evidence of any material contamination from these sources, but it was obvious that, if the persons in charge of this part of the work had been careless, gross contamination might have occurred, and it is probably owing to such contamination that many of the samples purchased on the market owe the number of bacteria found in them. It appears to me, therefore, that our results show that carefully prepared condensed milk should contain much fewer bacteria than are generally found in samples purchased on the market. It would be difficult for the makers to produce a saleable article if they did not attend with great care to the heating and evaporation of the milk. It is therefore probable that up to the time when the process of condensation is completed the work is conducted with great care in most conden-



DIAGRAM 3. Lesions produced in two Guinea Pigs by Tuberculous Milk heated to 77° C and 92° G respectively, and in one Guinea Pig (control) by the same Milk not Pasteurised. Each Guinea Pig is represented 7 times to show the lesions observable after various intervals in the course of 78 days.

• Lesions felt during life. ● Lesions observed after death. ○ Lesions which would probably have developed if the animals had lived.

series, but I believe that *from a commercial point of view* the same amount of care is not necessary after the actual process of condensation has been completed. The only thing that appears necessary for the purpose of obtaining a commercially satisfactory article is stirring of the cooling milk at a certain rate for a sufficient length of time. Bacteria introduced at this stage by dirty hands, dirty utensils, dust, water or tins, would probably not be able to multiply in the thick syrupy material and would only show their presence after the milk had been reconstituted by dilution.* It appears to me that the crude methods used at present for stirring, cooling, &c., may give opportunities for contamination, which can only be avoided by great care and cleanliness.

Inoculation tests of the various products used for infecting the 50 gallons of milk.

Tuberculous cow's milk.—An account of the tests carried out between December 7th, 1909, and February 20th, 1910, has been given at page 2.

Cultures of tubercle bacillus 128. (2,132A.) Guinea pig inoculated subcutaneously in the left leg with 1 c.c. of infected milk, prepared by suspending a potato culture 23 days old of bacillus 128, in 140 c.c. of sterilized cow's milk. After seven days there was a distinct local and corresponding inguinal swelling; after 14 days the local swelling was found to have spread considerably, the left superficial inguinal glands were much enlarged, the right slightly so; 21 days after inoculation the animal was killed, there was a large local lesion at the seat of inoculation, considerable induration and infiltration of the adductor muscles, considerable enlargement and caseation of the left popliteal, superficial inguinal, deep inguinal and sacro-lumbar glands. The retrohepatic ganglion was in the same state. The right superficial inguinal and sacro-lumbar ganglia were also affected, but much less than the left, the spleen and the liver contained a few tuberculous nodules. Tubercle bacilli were abundant in these lesions. The other specimens were tested in the same way. The records of results have been summarised in Table II., p. 14. Diagram 3 shows graphically the distribution of the lesions in three of the guinea pigs (one inoculated with untreated tuberculous milk and two with the same milk after treatment).

Feeding tests.—These tests will be discussed with those to which the dry milk was submitted (p. 46). None of the four rabbits fed on the treated tuberculous milk contracted tuberculosis.

* *On the Bearing of Outbreaks of food poisoning upon the Etiology of Epidemic Diarrhœa*—Author's reply at the end of the discussion on the paper—Transactions of the Epidemiological Society of London. N.S. Vol. XXII. 1902-1903.

TABLE II.—*Condensed*

Tabulated summary of the most salient results of the inoculation tests
o means no *obvious* lesion, † slight lesions, †† well marked

Laboratory books reference Nos.	No. of sample in text of report.	Nature of samples tested.	Quantity of material injected.	Duration of experiments.
			C.C.	Days.
2119 A	1	<i>Mixed milk on its arrival at the factory, before admixture with infective material.</i> 50 gallons used in experiment with tuberculous milk ...	40	46
2119 B	1	Do. do. do.	40	33
2123 A	5	Mixed milk from 1,000 gallons tank ...	40	78
2123 B	5	Do. do.	40	12
2124 A	6	Same milk as above (spec. 5) after passage through heater (2nd method) and heating to 86° C.	40	43
2124 B	6	Do. do. do.	40	78
2132 A	—	<i>Tuberculous infective material used in the experiments.</i> Potato culture of Bacillus 128 (23 days at 37° C.) mixed with 140 c.c. of sterilised milk.	1	21
2132 B	—	Potato culture of Bacillus 128 (47 days at 37° C.) mixed with 140 c.c. of sterilised milk	1	21
2133 A	—	Milk culture of Bacillus 128 (23 days at 37° C.)... ..	1	22
2133 B	—	Glycerine milk culture of Bacillus 128 (23 days at 37° C.)	1	22
2134 A	—	Milk from the 4 quarters of the tuberculous udder of cow. (M.B. 8747.)	1	23
2120 A	2	<i>Mixture of tuberculous infective material with 50 gallons of ordinary milk—used in experiment.</i> Milk taken from churn just before treatment (11.15 a.m.)	40	43
2120 B	2	Do. do. do.	40	13
2121 A	3	<i>Tuberculous milk during and after treatment.</i> After pasteurisation at 77° C. (11.35 a.m.)	40	6
2121 B	3	Do. do. do. do. do.	40	78
2122 A	4	After heating up to 92° C. (11.45 a.m.)	40	78
2122 B	4	Do. do. do. do. do.	40	47
2125 A	7	After addition of sugar (12.57 p.m.)	40	50
2125 B	7	Do. do. do. do. do.	40	78
2126 A	8	Condensed, just finished, taken from outlet of vacuum pan, reconstituted by adding 2 parts of sterile water to 1 part of condensed milk (1.14 p.m.).	40	78
2126 B	8		40	78
2127 A	9	Condensed milk taken from cooling cylinder after cooling for 1 hour 20 minutes (2.34 p.m.).	40	51
2127 B		Do. do. do.	40	78

*N.B.—When the quantity of milk tested exceeded 2 c.c. the milk was centrifugalised and sample a separate syringe previously sterilised by steam under pressure was used.

It will be noticed that the natural tuberculous milk obtained direct from the
with cultures on

** See description of Author's method—"The value of experimental

Milk (Method A.)

(guinea pigs inoculated subcutaneously on the inner side of left knee).**

lesions, ††† extensive lesions, — no observation.

Local and lymphatic lesions due to the inoculations, and recognisable during the life of the animal.						Macroscopical lesions observed, post-mortem in certain lymphatic glands and other organs (in which tubercle bacilli were found microscopically).					Non-tuberculous lesions observed post-mortem (lesions in which no tubercle bacilli could be found).		
2nd week.	3rd week.	4th week.	6th week.	8th week.	10th week.	Skin and subcutaneous tissues of left leg	Left inguinal glands.	Left sublumbar and sacral glands.	Spleen.	Liver.	Lungs.		
††	†	?	?	—	—	o	o	o	o	o	o	Slight induration of left inguinal glands.	
††	†	?	—	—	—	o	o	o	o	o	o	Slight local induration.	
†††	††	†	†	†	†	o	o	o	o	o	o	Scar of local ulcer.	
†††	—	—	—	—	—	o	o	o	o	o	o	Large local abscess and softening of corresponding lymphatic glands.	
†	o	o	o	o	o	o	o	o	o	o	o	—	
†	o	o	o	o	o	o	o	o	o	o	o	—	
††	†††	—	—	—	—	†††	†††	†††	†	†	o?	—	
†	††	—	—	—	—	††	†††	†††	†	o	o	—	
†	††	—	—	—	—	††	†††	†††	†	o?	o	—	
†	††	—	—	—	—	††	†††	†††	†	o	o	—	
††	†††	—	—	—	—	†††	†††	†††	††	††	†	—	
††	†††	†††	†††	—	—	†††	†††	†††	†††	†††	†††	—	
††	—	—	—	—	—	†	††	o	o	o	o	Acute peritonitis.	
†	—	—	—	—	—	?	o	—	—	—	—	Accidental death, slight local lesion.	
†	o	†	†	†	†	o?	†	†	†	o	o	Slight local induration.	
†	o	o	†	—	—	o?	†	†††	†††	†††	††	Slight local induration.	
†	o	o	o	—	—	o	o	o	o	o	o	Slight local induration.	
?	o	o	o	o	o	o	o	o	o	o	o	—	
†	††	††	††	†	o	o	o	o	o	o	o	Induration at seat of inoculation	
o	o	o	o	o	o	o	o	o	o	o	o	Abscess of mesenteric and suprasternal glands (food infection).	
†	††	††	††	—	—	o	o	o	o	o	o	Gastro-enteritis (food infection).	
†	††	††	††	o	o	o	o	o	o	o	o	Slight enlargement of popliteal, inguinal and sublumbar glands.	

the sediment mixed with 2 c.c. of the separated milk was used for inoculation. For each The inoculated animals were isolated in separate sterilised cages. tuberculous udder was slightly more infective than the mixtures prepared artificial media.

Tuberculosis in Diagnosis." (British Medical Journal II. p. 665, 1893).

Summary of results of inoculation tests.

The results recorded in the table show:—

1. That, before admixture with tuberculous milk, the fresh milk received at the factory did not on the day of the experiment contain an appreciable number of tubercle bacilli, but that other bacteria were present which caused local inflammatory lesions when injected under the skin of guinea pigs.

2. That the heating of this milk to 86° C. reduced to a considerable extent this pathogenic action.

3. That a mixture of one part of milk rich in tubercle bacilli with 108 parts of the above non-tuberculous milk produced rapidly extensive tuberculosis in guinea pigs inoculated with it.

4. That after the same mixture had been heated to 77° C. it was still capable of producing tuberculosis, but much more slowly and less extensively than the untreated mixture.

5. That after the mixture had been further heated to 92° C. it was still capable of producing tuberculosis, resembling in type that produced by milk heated up to 77° C.

6. That none of the sweetened condensed milk prepared with the infective mixture produced tuberculosis in the inoculated animals, but that some of the samples caused inflammatory lesions not produced by the pasteurised milk before sweetening and condensing.

7. That none of the rabbits fed with the re-constituted condensed milk contracted tuberculosis. (For details, see Section VIII. of this report.

III. OBSERVATIONS AND EXPERIMENTS RELATING TO METHOD B.

(DRYING OF MILK OVER HEATED REVOLVING CYLINDERS.)

Short description of the method.

On February 10th, 1910, Dr. Coutts visited Factory X and on July 12th, 1911, Dr. Coutts and I visited Factory Y. At both places Method B was used on a commercial scale. The following short description is based upon the observations which I had the opportunity of making on the second occasion:—

1. The fresh milk after being weighed is poured into a tank. As the milk passes from this tank to the drying cylinders its flow is regulated.

2. The drying apparatus consists of two cylinders (see Diagram 4), 150 cm. in length and 75 cm. in diameter. These two cylinders are placed side by side, the space between them not exceeding two millimetres. They rotate (at the rate of 6 to 14 revolutions per minute in opposite directions. (See Diagram.) They are heated by steam at a pressure of 38 to 40 lbs. (temperature 139° to 141° C.) when whole milk is treated, or 27 to 30 lbs.

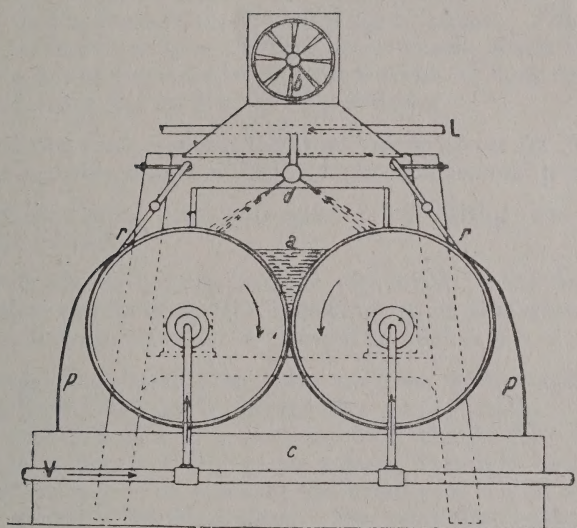


DIAGRAM 4.

(temperature 131° to 134° C.) in the case of separated milk. The steam is admitted to the interior of the cylinders by axial pipes (branches of pipe V in diagram).

It is in the gutter (*a* in diagram) formed by the adjacent upper surfaces of the cylinders that the milk is delivered from the tank by pipe *d* (the arrangement at Factory Y was slightly different from that shown in the diagram).

During the short time the milk remains in the gutter it passes into a state of ebullition owing to the escape of large bubbles of steam (mixed with the gases held by the milk). By means of a thermometer, the bulb of which was brought within 2 mm. from the surface of the cylinders at a place where the fluid was deepest, I ascertained that the temperature of the milk in the gutter oscillated between 98.5° and 99° C.

3. Below the slit at the bottom of the gutter a thin layer of milk remains on the surface of each rotating cylinder. This milk dries rapidly in the form of a thin pellicle, which, when the cylinder has made about two-thirds of a revolution, is firm enough to be separated from the surface of the cylinder.

4. The dry film is separated from the cylinder by long straight blades (*r.r.* in the diagram) in the form of an almost perfect ribbon.

5. The separated film falls into a collecting box (C) placed under the cylinder.

6. From the collecting box the dry milk, in the form of large thin scales, produced by the breaking up of the ribbon, is transferred to barrels where it is allowed to cool.

7. From the barrels the dry milk is, when cold, gradually reduced to fine powder by means of a special mill.

This mill consists of a covered box, the bottom of which is formed by a fine metallic gauze screen, through which the milk is rubbed by means of a brush, the bristles of which are arranged spirally round a rotating stem. The powdered milk, after passing through the screen, is guided by a hopper into a receiving box.

8. The milk powder is then packed into suitable receptacles for purposes of sale.

By this method it is possible, on an average, to dry about 65 gallons of whole milk per hour with one of the machines described above.

Description of first experiment (Factory X). (Drying of milk infected with tubercle bacilli.)

Nature of samples.—After some separated milk had been treated the tuberculous milk (10 gallons) was poured into the tank.

Previous to this, two samples of this milk (*Samples 1 and 2*) had been taken.

Two more samples (*Samples 3 and 4*) of the untreated tuberculous milk were taken from the tank when it was nearly empty,

i.e., when nearly the whole of the milk had been dried. Some of the dry milk was collected in sterilized tins, as it separated from the cylinders at an early stage.

One sample (*Sample 5*) was taken from one cylinder.

A second sample (*Sample 6*) was taken from the other cylinder.

Two more samples of dry milk were taken in the same way, one from each cylinder when the operation was nearly completed (*Samples 7 and 8*).

The greater part of the dry tuberculous milk was afterwards packed in metal tins and forwarded to the laboratory (*Samples 9 and 10*).

Description of the samples.

The infected untreated milk (*Samples 1, 2, 3 and 4*) was very dirty, a heavy sediment of coarse yellowish and black granules formed a deposit at the bottom of the bottles. The sediment was more abundant in the samples 3 and 4 taken from the tank towards the end of the operation than in samples 1 and 2 taken at the beginning. It was very easy to demonstrate microscopically the presence of tubercle bacilli in the sediment obtained from 10 c.c. of this infected milk.

The dried milk was in thin, very light and brittle flakes of a creamy colour containing only 2.43 per cent. of water. It had a faint acid reaction to litmus after being reconstituted with distilled water. The reconstituted milk yielded a heavy sediment similar to that obtained from the untreated milk. It contained in addition many brown particles. The brown particles were fragments of the parts of the pellicle which had been overheated owing to adhesion to the cylinders. I spread some of the flakes on cover glasses, moistened them, dried them again, fixed them in the usual way, and, after staining them according to the Ziehl Neelsen method, found that they all contained a fairly large number of unaltered cells, and that flakes measuring about half inch in diameter generally contained tubercle bacilli; clusters of these bacilli were present in several of the flakes examined.

Cultivation tests.

Aerobic and anaerobic bouillon cultures of the infected milk (*Sample 1*) and of the dry milk (*Sample 5*) were made each with 1 c.c. of milk (the dry milk was reconstituted by mixing it with sterile water (one part of dry milk to six parts of water)).

In both cases the presence of aerobic and anaerobic sporing bacilli was clearly demonstrated. Aerobic bouillon culture, made with 1/100th gramme of dry milk, contained almost nothing else than cocci (diplococci, streptococci, tetrads and sarcinae).

Aerobic gelatine and agar plate cultures were made with dilutions corresponding to 1/100th, 1/10,000th, 1/100,000th and 1/1,000,000th cubic centimetres of the original infected milk (*Samples 1 and 3*) and with dilutions corresponding to 1/700th,

1/70,000th, 1/700,000th, 1/7,000,000th grammes of the dry milk (Samples 5 and 7) with the following results:—

Aerobic gelatine plates (72 hours incubated at 20° C.).

Infected milk before treatment,	
average total number of colonies ... per 1 c.c.,	104,900,000
Infected milk after drying (reconstituted)	4,900
Infected milk after drying (and keeping for 112 days)	4,180

The proportion of liquefying colonies to non-liquefying colonies was in the case of the untreated milk about 1 to 6; in the case of the dried milk about 1 to 20 in one case, 1 to 50 in another case.

Aerobic litmus lactose agar plates (72 hours at 37° C.).

Infected milk before treatment,	
average total number of colonies ... per 1 c.c.	7,200
Infected milk after drying (reconstituted)	800
Infected milk after drying (and keeping for 112 days)	500 to 600

The most characteristic organisms found in the agar plates prepared with dry milk were those of bacilli having the characters of the *B. mesentericus vulgatus* and of the *B. mesentericus ruber*. A few acid producing colonies were found, but none were colonies of bacilli of the *B. coli* group.

Inoculation tests.

These experiments may be conveniently divided into three groups:—

(a) Testing of the various products employed to infect the ten gallons of milk.

(b) Testing of samples taken from the ten gallons of milk after infection but before treatment.

(c) Testing of the dry milk prepared with the infected milk.

The results of these tests are summarised in Table III., and show that the process does not entirely destroy the virulence of tubercle bacilli.

Feeding tests.

None of the four rabbits fed on dried tuberculous milk contracted tuberculosis. Fuller particulars of these experiments are given in Section VIII. of this report (p. 46).

TABLE III.—*Dry Milk.*

Tabulated summary of the most salient results of the inoculation tests

o means no *obvious* lesions, † slight lesions, †† well marked

Laboratory books reference Nos.	No. of sample in text of report.	Nature of samples tested.	Quantity of material injected.	Duration of experiments.
			C.C. or grammes	Days.
		A.— <i>Tuberculous infective material used in the experiments.</i>		
2134 A	—	Milk from the 4 quarters of the tuberculous under (M.B. 8747.)	1	23
2115 A	—	Milk and potato cultures of Bacillus 128 mixed...	1	23
2115 B	—	Mixture of milk and potato culture of Bacillus 128 and of tuberculous milk from tuberculous udder. (M.B. 8747.)	1	23
2116 A	—	Culture of tubercle bacilli 128 in plain milk, 36 days at 37° C.	1	23
2116 B	—	Culture of tubercle bacilli 128 in glycerine milk (5%) 36 days at 37° C.	1	23
		B.— <i>Mixture of tuberculous infected material with 10 gallons of ordinary milk, used in experiment.</i>		
2113 A	1	Taken from churn just before beginning of experiment	40	4
2113 B	2	Do. do. do.	40	24
2114 A	3	Taken from tank of untreated milk towards end of operations.	40	24
2114 B	4	Do. do. do.	40	24
		C.— <i>Tuberculous milk after drying and reconstitution by addition of sterile water.</i>		
		15.6 c.c. of reconstituted milk corresponding to 2 grammes of dry milk.		
2117 A	5	Dry milk collected from one roller at the beginning of operation.	2 grammes.	79
2117 B	6	Dry milk collected from other roller at the beginning of operation.	2 „	35
2118 A	7	Dry milk collected from one roller towards the end of operation.	2 „	30
2118 B	8	Dry milk collected from other roller towards the end of operation.	2 „	14

* N.B.—When the quantity of milk tested exceeded 2 c.c. the milk was centrifugalised and sample a separate syringe previously sterilised by steam under pressure was used.

It will be noticed that the natural tuberculous milk obtained direct from the with cultures on

(Method B.)

(guinea pigs inoculated subcutaneously on the inner side of left knee).*

lesions, ††† extensive lesions, — no observations.

Local lymphatic lesions due to the inoculations and recognisable during the life of the animal.							Macroscopical lesions observed post-mortem in certain lymphatic glands and other organs (in which tubercle bacilli were found microscopically).						Non-tuberculous lesions observed post-mortem (lesions in which no tubercle bacilli could be found).
2nd week.	3rd week.	4th week.	5th week.	6th week.	8th week.	10th week.	Skin and subcutaneous tissues of left leg.	Left inguinal glands.	Left sublumbar and sacral glands.	Spleen.	Liver.	Lungs.	
††	†††	—	—	—	—	—	††	†††	†††	††	††	†	o
†	†††	—	—	—	—	—	†††	†††	†††	††	†	—	o
††	†††	—	—	—	—	—	†††	†††	†††	†††	††	†	o
†	†††	—	—	—	—	—	††	†††	†††	††	†	—	o
†	†††	—	—	—	—	—	††	†††	†††	††	†	—	o
—	—	—	—	—	—	—	—	—	—	—	—	—	Accidental death.
††	†††	—	—	—	—	—	†††	†††	†††	†††	o?	—	—
††	†††	—	—	—	—	—	†††	†††	†††	†††	o?	—	—
††	†††	—	—	—	—	—	†††	†††	†††	†††	o?	—	—
††	†?	o	o	†	††	†††	††	††	†††	†††	†††	†?	—
†	†?	o	o	—	—	—	†	††	††	o	o	o	—
†††	††	††	—	—	—	—	††	o	o	o	o	o	—
††	—	—	—	—	—	—	††	o	o	o	o	o	Accidental death.

the sediment mixed with 2 c.c. of the separated milk was used for inoculation. For each The inoculated animals were isolated in separate sterilised cages. tuberculous udder was slightly more infective than the mixtures prepared artificial media.

IV. METHOD B. DRYING OF WHOLE AND OF SEPARATED MILK OVER HEATED REVOLVING CYLINDERS. (FACTORY Y.) (SECOND SET OF EXPERIMENTS.)

Description of experiments. Nature of samples.

1. *Drying of whole milk.*—A can containing 17 gallons of milk which had been brought from a good farm the same morning between 7.30 and 8.30 a.m. was placed at our disposal.

After stirring the milk thoroughly with the metal plunger generally used for this purpose, I collected (11 a.m.) a sample of this mixed milk (*Sample A*).

This sample was immediately placed in a portable refrigerator to prevent any further multiplication of bacteria.

At 11.7 a.m. the can was emptied into the distributing tank, the drying operation was proceeded with immediately, and the 17 gallons of fresh milk were converted into dry milk in 13 minutes.

At 11.20, *i.e.*, about half a minute before the end of the operation, I collected a sample of the dry milk as it fell from the hot cylinder (*Sample B*).

In order to prevent contact between this milk and any unsterilized object, I directed part of the falling pellicle by means of two sterilized rods into a sterilized tin, which was immediately hermetically sealed with a sterilized lid.

Without allowing the dry milk to remain as long as usual in the wooden barrel in which it is allowed to cool, some of that material was transferred to the powdering mill, under the hopper of which I placed a sterilized tin to receive directly some of the milk powder (*Sample E*.) After a sufficient amount of powder had accumulated the tin was closed hermetically with a sterilized lid.

2. *Drying of separated milk.*—Seventeen gallons of separated milk were treated in the same way as the whole milk had been.

This milk was part of a consignment which had arrived from country farms the same morning between 7 and 8 o'clock, and had since then been separated and passed over a refrigerator cooled by a current of water.

At 11.37 I took a sample of this separated milk (*Sample C*).

The contents of the 17 gallons can were then poured into the regulating tank of the drying machine and treated forthwith.

At 11.45, *i.e.*, eight minutes after the beginning of the drying operation, a sample of the dry milk (*Sample D*) was taken as it fell from the rollers. The same precautions as those previously mentioned were taken to prevent any accidental contamination.

During this operation the cylinders were heated by steam at a pressure of 27 to 30 lbs. (temperature 131° C. to 134° C.).

I noticed that as it fell from the cylinder the ribbon of dry separated milk was softer and less brittle than the ribbon of dry whole milk.

Some features of the samples.—The characters of the samples obtained in the course of this set of experiments were similar to those of the samples described in Section III. of this report, with that difference that the milk was less dirty, poorer in cells, and



DIAGRAM 5. Lesions produced in two Guinea Pigs by Tuberculous Milk dried according to method B, and in one Guinea Pig (control) inoculated with the Tuberculous Milk before treatment.

(For explanations see Diagram 3 and text.)

quite free from tubercle bacilli. It contained also a smaller amount of the brown particles indicating the effects of over-heating.

The total amount of solids non-volatile at 100° C. and the reaction of the original and of the dry milk after reconstitution with sterile pure water, are given in the following table:—

Samples.	Reaction of original milk and of treated milk after reconstitution with pure water.	Total solids non-volatile at 100° C. Per cent.	Approximate quantity of original milk corresponding to 1 gramme of dry milk.
A. Original whole milk ...	Amphóterous	13.95	1
B. Dry milk from cylinder	Very faintly acid	96.11	6.88
E. Dry milk powdered ...	„ „	96.68	6.93
C. Original separated milk	Acid	8.87	1
D. Dry separated milk	Slightly acid	92.53	10.43

The ratios given in the last column to the right were used to correct the figures indicating the number of bacteria found in the quantities of dry milk used for plate cultures. This was necessary in order to estimate with reasonable accuracy the changes occurring in the number of bacteria at various stages of the process.

Cultivation tests.

1st set.—Measured quantities of each of the samples were incubated in *peptone bouillon* at 37° C. These cultures were kept under observation for 17 days, any evidence of growth, *e.g.*, cloudiness, formation of sediment or pellicle, were noted and controlled by microscopical examination and sub-cultivations on ordinary media.

2nd set.—Two loopfuls of the contents of each of the above bouillon cultures were spread over the surface of litmus lactose agar plates, which were incubated for three days at 37° C. and at 16° to 18° C. for 14 days more. These plates were examined on several occasions in the course of three weeks, and each colony showing characteristic features sub-cultured and examined microscopically.

3rd set.—*Litmus lactose agar plates* were prepared with measured quantities of the samples themselves, incubated and kept under observation as those of the second set.

4th set.—*Glucose agar plates* were prepared as those of the third set and incubated anaerobically at 37° C.

5th set.—*Peptone bouillon gelatine plates* were prepared with measured quantities of the various samples and incubated at 18° to 20° C. These plates were used chiefly to estimate the number of bacteria growing at room temperature.

All these tests yielded interesting results with the exception of the fourth, which gave results differing but little from those yielded by the third, probably owing to the small number of strict anaerobic bacteria.

Some of the samples of dry milk appeared to be sterile when the amount used did not exceed $1/100$ th or $1/10$ th of a gramme. In those cases the tests were repeated with 1 gramme and 5 grammes of milk respectively. These quantities of dry milk were mixed with a sufficient amount of water and then incubated. I did not find it convenient to make plates with more than $1/10$ th gramme of dry milk to one plate owing to the opacity produced by the insoluble parts of the milk when the amount of dry milk exceeded that quantity. A microscopical examination of all the fluid cultures was made to ascertain by direct observation the predominant types of bacteria capable of multiplying rapidly in a fluid medium at blood temperature. The agar plates prepared with these fluid media (second set) were used for the same purpose and to differentiate the kinds of bacteria which had grown in bouillon.

The third and fifth sets of plates were used to estimate the number of, and to isolate the most prevalent types of bacteria actually present in each sample, and the characters of pure cultures of these bacteria were ascertained afterwards.

About 200 primary plates or cultures and more than 300 sub-cultures were prepared and kept under observation for two or three weeks on an average.

A complete record of all these observations would occupy a considerable space. I have therefore tabulated the results in Tables IV., V., VI. and VII., so as to make them easily comparable. In preparing these summaries I have been obliged to eliminate all the observations which did not seem of special interest or which had to be made only for purposes of control.

To exclude as far as possible the error due to the unequal distribution of bacteria, more specially in milk reconstituted from dry milk, all the original dilutions were prepared with quantities of material exceeding 1 gramme in weight. Each dilution was shaken violently in the shaking machine for half an hour before being used to prepare higher dilutions, plates, or other cultures. Even after this treatment I was not satisfied that the bacteria were equally distributed in the fluid, and to eliminate false estimates due to the occurrence of small clumps of bacteria in the high dilutions, I have generally discarded plates which were not either sterile or not showing at least 20 discrete colonies. As four sets of plates were made with each dilution, the results recorded in the tables may be accepted as giving a fair estimate of the number of bacteria capable of growing under the conditions which I have mentioned. It must, however, be remembered that there are in milk some micro-organisms which are not revealed by these methods; this is indicated by the fact that many of the plates which appeared at first to be free from streptothrichae were found to harbour a fair number of these organisms after they had been kept at room temperature for more than one week. The vital concurrence between the milk bacteria is also a disturbing

factor. In bouillon cultures, and even in agar cultures in which colonies are crowded, staphylococci and streptococci may mask at first the presence of certain bacilli, many of the cocci render the medium very acid. This is well shown in litmus lactose agar plates, which may become uniformly red during the first four or five days, after which colonies of bacilli and of some cocci producing a strong alkaline reaction may appear among the acid producing colonies, and after a few more days the plates may have a uniformly blue colour. The effects of that vital concurrence upon the relative number of various kinds of bacteria could not be allowed for without a considerable expenditure of time. It is well to remember the existence of this phenomenon and not to attach undue importance to numerical estimates obtained by the usual methods. When comparing, for instance, fresh milk with milk which has been heated, one finds that cocci and small bacilli predominate in the fresh milk to such an extent that some of the larger sporing bacilli appear to be in insignificant numbers. In the heated milk on the contrary one generally finds few or no cocci, and sporing bacilli take the leading place. The cocci reappear when the prepared milk has been handled.

A short account of the bacteria and allied organisms isolated from the dry milk and from the original milk is given in Section VI. of this report (p. 33).

Opportunities for contamination during manufacture of dry milk.

The box into which the ribbon of dry milk was collected as it fell from the cylinders was very near the floor and not protected against dust. The flocculent mass was frequently pressed down by the hands of an operator who had previously been handling fresh milk, milk cans arriving from the farm and other articles exposed to pollution. This operator wore his ordinary clothes. No special provision had been made for the washing of hands, &c.

The receptacles in which the dry milk was allowed to cool were large wooden barrels, made of thin, not well joined, rough, staves; it would have been practically impossible to prevent accumulation of dust in such barrels or to sterilize them.

In transferring the dry milk from the collecting box to the barrel and from the barrel to the powdering mill, a large scoop with a short handle was employed. In order to use this scoop the operator had to plunge his hand and part of his arm into the dry milk.

TABLE IV.—Method B. Second set of experiments. Whole Milk.

Cultivation Method.	Sample.	Quantity of original material in grammes.							Number of bacteria per gramme, average.
		1.0	0.1	0.01	0.001	0.0001	0.000001		
Neutral peptone bouillon ... (48 hrs. at 42° C.)	A. Whole milk, fresh	—	—	—	+++	—	—	10,000 to 100,000	
	B. Whole milk, dry from roller ...	+++	+++	0	0	—	—	10 to 100	
	E. Whole milk, dry powdered ...	—	—	+++	+++	0	—	1,000 to 10,000	
Litmus lactose agar ... (48 hrs at 37° C.)	A. See above	—	—	—	485	78	5	588,300	
	B. "	—	—	0	0	0	—	?	
	E. "	80	6	69	5	0	—	70 5,950	
Glucose agar... 24 hrs anaerobic (24 hrs aerobic, 37° C.)...	A. See above	—	—	—	310	25	0	280,000	
	B. "	—	—	3	0	0	—	300	
	E. "	—	—	61	8	0	—	7,050	
Peptone bouillon gelatine ... (4 days 18° to 20° C.)	A. See above	—	—	—	455	14	2	265,000	
	B. "	—	—	1	0	0	—	100	
	E. "	—	—	202	9	0	—	14,600	
	+++ Abundant growth.	o No growth.							— No observation.

+++ Abundant growth.

0 No growth.

— No observation.

TABLE V.—*Method B. Whole Milk.*

Estimated number of colonies per gramme, as revealed by methods used.

SAMPLES.	Number of bacteria in 1 gramme of material.		Number of bacteria in 1 cubic centimetre of original milk, or of reconstituted dry milk.	Approximate weight of original milk corresponding to 1 gramme of treated milk.
	Maximum 4 methods.	Minimum 4 methods.	Maximum No.	
A. Whole milk taken from the can on arrival from the farm ..	588,000 ⁽¹⁾	265,000 ⁽³⁾	588,300	1.00
B. Dry milk collected as it fell from the hot roller	300 ⁽²⁾	70 ⁽³⁾	43	6.88
E. Dry milk, still warm, collected as it fell from the powdering mill	14,600 ⁽³⁾	5,950 ⁽¹⁾	2,106	6.93

(1) Litmus lactose agar.

(2) Glucose agar.

(3) Gelatine.

TABLE VI.—Method B. Separated Milk.

Cultivation method.	Sample.	Quantity of original material in grammes.						Number of bacteria per gramme. Averages
		1.0	0.1	0.01	0.001	0.0001	0.000001	
Neutral peptone bouillon. (48 hrs. at 42°) ...	C. Separated milk before treatment D. Separated milk dry from roller.	— ††	— o	— o	††† o	††† o	†† —	More than 1,000,000 1 to 10
Litmus lactose agar. (48 hrs. at 37° C.)	C. See above C. " 1 2	— — 110	— — 9	— 1 —	∞ o —	∞ o —	54 — —	38,800,000 100
Glucose agar ... 24 hrs anaerobic... (24 hrs. aerobic at 37° C.)	C. See above D. " ...	— —	— —	— spoilt.	∞ o	∞ o	34 —	34,000,000 ? (less than 100.)
Peptone bouillon gelatine. (4 days at 18° C. to 20° C.)	C. See above D. " ...	— —	— —	∞ o	∞ o	500 o	31 o	3,700,000 (less than 100.)

Explanation of signs :—
 †† Growth moderate.
 ††† Growth abundant.

∞ Number of colonies uncountable.
 — No observation,
 o No growth.

TABLE VII.—*Method B. Separated Milk.*

Estimated number of colonies per gramme, as revealed by methods used.

SAMPLES.	Number of bacteria in 1 gramme of material.		Number of bacteria in 1 cubic centimetre of original milk, or of reconstituted condensed or dry milk.	Approximate weight of original milk corresponding to 1 gramme of treated milk.
	Maximum 4 methods.	Minimum 4 methods.	Maximum No.	
C. Separated milk collected shortly after separation.	(1) 38,800,000	(2) 3,700,000	38,800,000	1.00
D. Dry separated milk collected as it fell from the hot roller	(1) 100	(3) 10?	9	10.43

(1) Litmus lactose agar.
(2) Gelatine.

(3) Bouillon.

The construction of the powdering mill was favourable to the accumulation of dust. Disinfection of that appliance (for which no provision had apparently been made) would have been a matter of great difficulty. The box into which the powdered milk was collected was very close to the floor, and all the final operations were conducted in a room where milk cans arriving from the farm were received and where also the milk was separated and cooled. The floor was exposed to soiling by the boots of men coming into it from outside.

V. OBSERVATIONS AND EXPERIMENTS RELATING TO METHOD C.

(Drying by spraying the milk into a current of hot air).

Short description of the method. (Diagram 6).

Dr. Coutts and I visited Factory Z on July 13th, 1911. The preparation of dry milk as conducted at that place may be briefly described as follows:—

1. The milk on arriving from the farm is poured into a large vessel the outlet of which is provided with a strainer; this vessel is also used to weigh the milk.

2. The strained milk is allowed to mix in a large tank from which it is admitted to

3. a heater, where it is warmed before passing to

4. the separator. The separated milk may be dried separately, in which case the cream is removed. When dried whole milk is wanted the separated milk and cream are brought together again in a common channel which carries the milk to

5. a pasteurising apparatus where the temperature of the fluid is raised from 70° C. to 75° C. When separated milk is treated the temperature is raised slightly above 75° C.

6. The pasteurised milk is collected in large tin-lined tanks the capacity of which is about 800 gallons.

7. The fluid is then carried by pipes to a large vacuum pan, where it is heated by steam to a temperature of about 58° C. and is reduced to a little less than one-half of its original bulk. When the milk has reached a suitable consistency its temperature is rapidly raised to 95° C., and as soon as this point is reached the temperature is brought down again to 58° C.

According to the amount of milk in the vacuum pan the condensing process occupies from 2 hours to $2\frac{1}{2}$ hours.

8. The condensed milk is drawn into metal cylinders from which it is immediately pumped into

9. a small tank, from which the fluid passes into

10. a powerful pump, which forces it under a pressure of 2,000 to 3,000 lbs. through the

11. spraying apparatus by which a cone of finely sprayed milk is produced. This takes place in the middle of an opening to which a current of dry air heated to 115° C. is brought by

12. a large pipe. Both the hot air and the spray are projected into

13. a tin-lined chamber, the internal temperature of which is 76° to 81° C. The droplets of milk dry as they fall to the floor upon which they rapidly form a layer of granules of dry milk.

The current of hot air passes out of this first room into a second room where the particles which have not fallen in the first room are collected.

When the desired amount of milk has been dried the drying room is emptied through an opening in the floor, the milk powder is collected in

14. metal cylinders.

15. It is afterwards sifted through a fine gauze screen.

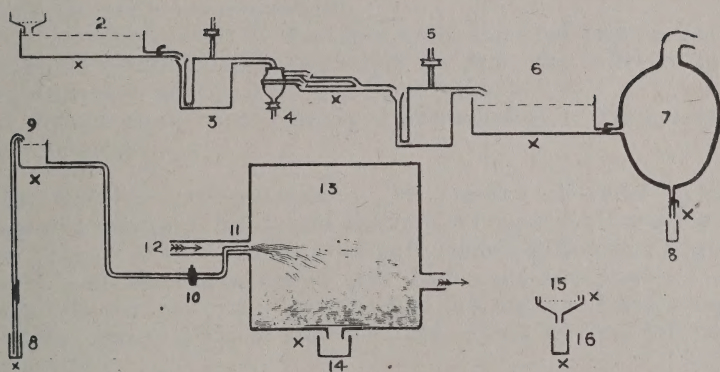
16. For wholesale purposes the finished product is packed in large bags of thin impervious paper supported by a wooden case.

For retail purposes the milk powder is packed in paraffined cardboard cups of various sizes.

About 6,000 gallons of milk could be treated daily at Factory Z.

Collection of samples.

At the time of our visit whole milk was being treated, and we timed the taking of the samples so as to secure as far as possible correspondence between the samples of untreated and of treated milk. (The samples of dried milk just finished were taken three hours after the samples of fresh milk had been collected.)



* Signs & Figures explained in text.

DIAGRAM 6.

The following samples were taken :—

At 9.25 a.m. a sample of the fresh mixed milk (*Sample O*) brought the same morning from various farms was collected at the outlet of tank 2.

10 a.m., *Sample P* was taken from the large tank (6) filled with pasteurised milk, the temperature of which was about 75° C. at the place where the sample was taken.

12 o'clock. A sample of the condensed milk (*Sample Q*) was taken at the outlet of the vacuum pan while the fluid was flowing into one of the metal cylinders (8).

12.30. A sample of milk powder just finished (*Sample R*) was abstracted from the drying chamber (13) in the following way :—

Before closing the chamber, a large sheet of clean new drawing paper, sterilized by a current of air heated to 115° C., had been laid on the floor of the chamber. As soon as it was thought that enough dry milk had accumulated on its surface, the sheet of paper was rapidly removed from the chamber and the milk made to slide quickly into a sterilized tin which was immediately closed hermetically with a sterilized lid.

A sample (*Sample S*) of the same milk, screened and packed in a paraffined cardboard box and ready for sale, was collected later in the day when all the milk had been treated.

All these samples were examined immediately on their arrival at the laboratory.

Some features of the samples.—The powder obtained by this process was very fine, light, and had a pale creamy yellowish white colour. It mixed well when thoroughly beaten with water slightly warmed, and the reconstituted milk had a pleasant taste. The reaction (to litmus) of the various samples and the total amount of solids non-volatile at 100° C. are given in the following summary :—

SAMPLES.	Reaction of original milk and of treated milk re-constituted with pure water.	Total solids non volatile at 100° C. Per cent.	Approximate weight of original milk corresponding to 1 gramme of treated milk
O. Original milk	Slightly acid	12.11	1.00
P. Pasteurised milk (after removal of slime) ...	Amphoterous	13.98	1.15
Q. Condensed milk	Slightly acid	36.36	3.00
R. Dry milk from drying chamber... ..	Very slightly acid ...	98.84	8.16
S. Dry milk after screening and packing	Very slightly acid ...	97.04	8.01

Cultivation tests.—The cultivation tests were conducted in the way described previously (Section IV.).

The results of these tests are summarised in Tables VIII. and IX.

TABLE VIII.—Whole Milk. Method C.

Cultivation Method.	Sample.	Quantity of original material in grammes.						Number of bacteria per gramme, averages.
		0.1	0.01	0.001	0.0001	0.00001	0.000001	
Neutral peptone bouillon, (48h. at 42° C.)	O.—Whole milk fresh	—	—	∞	+++	++	o?	100,000 to 1,000,000
	P.—" pasteurised	++	o	o	o	—	—	10 "
	Q.—" condensed	—	++	o	o	o	—	100 "
	R.—" dry from chambers	—	+++	+++	o	o	—	1,000 "
Litmus lactose agar, (48h. at 37° C.)	S.—" dry and packed...	—	+++	+++	++	o	—	10,000 "
	O.—(See above)	—	—	∞	561	67	5	5,770,000 (under 100)
	P.—" "	—	o	o	o	o	—	44,800
	Q.—" "	—	566	33	o	o	—	15,200
Glucose agar,— (24h. anaerobic 37°) (24h. aerobic 37°)	R.—" "	—	184	12	o	o	—	134,000
	S.—" "	—	∞	138	13	o	—	
	O.—(See above)	—	—	∞	∞	137	12	12,850,000 (under 100)
	P.—" "	—	o	o	o	o	—	9,900
Peptone bouillon gelatine, (4 days at 18° to 20° C.)	Q.—" "	—	158	4	o	o	—	14,500
	R.—" "	—	120	17	o	o	—	110,000
	S.—" "	—	∞	90	13	o	—	
	O.—(See above)	—	—	∞	726+	201	15	14,120,000
Explanation of signs :—	P.—" "	—	19	o	o	o	—	1,900
	Q.—" "	—	93	1	o	o	—	5,150
	R.—" "	—	112	9	o	o	—	10,100
	S.—" "	—	2220+	128	18	?	—	154,000

Explanation of signs :—

— means No observation.

o " Observation with negative results.

+ " Slight growth.

+++ means Moderate growth.

∞ " Abundant growth.

∞ " Number too large to be estimated accurately.

TABLE IX.—*Whole Milk. Method C.*
Estimated number of colonies per gramme, as revealed by
the methods used.

SAMPLES.	Number of bacteria in 1 gramme of material.		Number of bac- teria in 1 cubic centimetre of original milk, or of reconstituted condensed or dry milk.	Approximate weight of original milk corres- ponding to 1 gramme of treated milk.
	Maximum (4 methods)	Minimum (4 methods)	Maximum No. in 1 c.c. of original or of reconstituted milk.	
O. <i>Whole milk</i> taken from distributing tank on arrival from the farm	14,120,000 ⁽¹⁾	5,770,000 ⁽²⁾	14,120,000	1.00
P. <i>Whole milk</i> taken from tank of <i>Pasteur- ized milk</i>	1,900 ⁽¹⁾	100 ⁽²⁾	1,652	1.15
Q. <i>Condensed milk</i> col- lected at outlet of vacuum pan	44,800* ⁽²⁾	5,150 ⁽¹⁾	14,933*	3.00
R. <i>Dry milk</i> collected in hot drying chamber...	15,200 ⁽²⁾	10,100 ⁽¹⁾	1,862	8.16
S. <i>Dry milk sieved</i> and packed	154,000 ⁽¹⁾	110,000 ⁽³⁾	19,250	8.01

(1) Gelatine.

(2) Litmus agar.

(3) Glucose agar.

VI. TYPES OF LIVING MICROBES FOUND IN MILK PRESERVED ACCORDING TO METHODS A, B AND C.

In Section II. of this report reference has been made to the fact that condensed milk ready for sale almost invariably contained cocci and bacilli of several kinds, and that at all stages during the process of preparation sporing bacilli having the characters of the *B. mesentericus ruber* and of the *B. mesentericus vulgatus* were almost invariably present.

The first set of experiments was, however, devised chiefly to ascertain the fate of tubercle bacilli.

In the second set of experiments more attention was paid to the effects of the drying processes upon the various types of bacteria occurring in milk. The identification of non-pathogenic organisms isolated from treated milk was a matter of some difficulty owing to the alterations which heating and desiccation produced in the rate of growth and in other cultural characters of several kinds of bacteria. These alterations often made it impossible to establish a complete correspondence between the organisms isolated from our samples and those described (very incompletely, as a rule) by various workers.

These difficulties did not, however, interfere very materially with the chief object of the investigation, which was to ascer-

* This figure is based upon the actual observation, but is probably misleading. The minima figures indicate more accurately the relative number of bacteria in the various samples.

tain the fate of some of the bacteria found commonly in fresh milk and the source of those which were isolated from preserved milk in the form in which it is offered for sale.

The results obtained have been summarised in Tables X., XI. and XII.

TABLE X.

Types of microbes isolated from samples of whole milk, before, during and after treatment by Method B.

Morphological Types of Microbes.	Reaction of growths on litmus lactose agar plates.	Sample A. Original milk.	Sample B. Dry milk taken from the hot cylinders.	Sample E. Dry milk after passage through powdering mill.
Cocci.				
A. Small. 1. Staphylococci	Acid	+++	o	++
2. Streptococci	"	+++	o	+
B. Large—Sarcinae—white	Neutral or alk.	+++	o	+++
" " yellow	"	++	o	+
Bacilli.				
A. Small (non-sporing) ...	"	++	o	—
" B. Coli like ...	Acid	+++	o	—
B. Large, several kinds, (sporing)	Neutral or alk.	++	++	+
Streptothrichæ—white ...	Neutral	+++	+	++
Yeasts (large) ...	Acid	+	o	—
Moulds (several kinds) ...	—	—	o	+

† Few., ++ moderate number, +++ abundant, o none present after special search.
— This sign means that the organisms in question were very scanty if present, i.e., on ordinary examination of the plates no colonies were noticed, but no further steps were taken to control these first results.

TABLE XI.

Types of microbes isolated from samples of separated milk before and during treatment by Method B.

Morphological types of microbes.	Reaction of growth on litmus lactose agar plates.	Sample C. Separated milk before treatment.	Sample D. Separated milk dry taken from hot cylinders.
Cocci—small ...	Acid.	+++	o
" large ...	Neutral or alk.	++	†*
Bacilli—small, producing yellow pigment.	Neutral.	++	o
" small, coli-like ...	Acid.	+++	o
" large sporing ...	Neutral or alk.	++	+++
Streptothrichæ—white ...	Neutral.	++	+

* The presence of a coccus in one of the samples of dry milk collected directly as it fell from the hot cylinder was exceptional, cocci were not found in other samples of the same type. As special precautions were taken to avoid any contact with contaminated objects, it is probable that the presence of this coccus was due to the fall of some dust upon the material at the time of collection. The same explanation may be applicable to the Streptothrix. (Tables X. and XI.)

TABLE XII.

Types of microbes isolated from samples of whole milk before, during and after treatment by Method C.

Morphological types of microbes.	Reaction of growth on litmus lactose agar plates.	Sample O. Fresh whole milk arriving from the farms.	Sample P. Same milk after pasteurisation at 75° C.	Sample Q. Same milk pasteurised and partly condensed.	Sample R. Same milk removed from hot chamber immediately after drying.	Sample S. Same milk (dry) after screening and packing.
Streptococci, long ...	Acid	†††	o	†††	†††	††
" " short ...	"	†††	o	†††	†††	††
Staphylococci ...	"	†††	o	†	†††	†††
Sarcinae, white ...	Neutral ?	†††	o	—	†††	†††
" " pink ...	Alkaline	†††	o	—	†††	—
Bacilli, small coli like ...	Acid	††	o	—	††	†
" " large sporing ...	Acid or Alkaline	††	††	††	†	†
Streptothrichae, white ...	Neutral	†††	o	o	o	††
" " brown ...	"	††	+	—	††	††
Yeast, large white ...	Acid	—	o	—	—	—
Moulds, various ...	—	—	o	—	—	—

Explanation of signs, see Table X.

In the foregoing tables I have reduced the number of types of microbes to 14, but each type must be understood to include a number of kinds having in common the characters indicated in the tables. It must also be kept in mind that all the types observed have not been entered in these summaries. In the group *Staphylococci* I have included cocci measuring on an average micron. 0.6 to 0.7 as well as larger cocci measuring from micron. 0.8 to 0.9. Among the *streptococci* there were similar differences in the size of the individual cocci, in addition to which some of the kinds of streptococci were long, others were short. Among the latter some were often grouped as staphylococci.

The large cocci, measuring generally from micron. 1. to 1.2 in diameter, were generally arranged in sarcina-like groups, some of the non-chromogenic large cocci may, however, have been staphylococci, the *yellow and the pink sarcinae* were typical.

There were at least four kinds of *small bacilli* of sizes varying between micron. 0.4 to 0.5 (diam.) $\times$ micron. 1 to 5 (length), some were chromogenic, but most were non-chromogenic. Among the latter some fermented lactose and others did not.

The large sporing bacilli measured generally micron. 0.7 to 0.9 (diam.) $\times$ micron. 1.5 to 5 or more (length). Three kinds of these sporing bacilli were specially studied; they had respectively the characters generally associated with the *B. mesentericus vulgatus*, *B. mesentericus ruber* and *B. mesentericus niger* of authors. These names must not, however, be taken to indicate very close resemblance among the bacilli which bear them. They have, however, certain important properties in common.

The distribution of these bacteria in the milk before, during and after treatment indicated clearly that *many of them which had disappeared* while the milk was heated and protected from the access of dust and from contact with contaminated articles, *had reappeared* in the final stages of preparation when no longer protected against contamination.

Many of the bacteria found in preserved milk as found on the market, owe their presence to a process of *recontamination*.

Preserved milk is sometimes handled by persons who have previously handled the fresh milk; it is also exposed to the same sources of contamination (hands, clothing, soil, dust, water used to clean vessels, etc.), as the fresh milk. These facts are sufficient to account for the great resemblance between the bacterial flora of fresh and of preserved milk.* I thought, however, that it was desirable to test the accuracy of that explanation by ascertaining the thermo-lethal point of the most characteristic bacteria which I had isolated from the samples of condensed and of dry milk examined in the course of this investigation.

* Whatever rôle flies may play in the contamination of preserved milk at the homes of consumers, they can be entirely excluded as factors of contamination of the prepared products collected at the four factories inspected by us. During our visits flies were very scarce, and had they been numerous they would have had no opportunity to contaminate our samples.

VII. THERMO-LETHAL POINT OF BACTERIA FOUND IN PRESERVED MILK.

A. *Saprophytic Bacteria*.

In a first set of experiments, carried out in 1911, three days' old pure bouillon cultures of the bacteria tested were heated in a water bath and kept at a temperature of 80° C. for 20 minutes. The temperature was then allowed to rise to 98.5° , which it did in six minutes. The temperature was ascertained by means of reliable thermometers plunging in the cultures, which were constantly stirred. After the cultures had been kept at 80° for 20 minutes, 0.1 c.c. of each was withdrawn by means of a pipette, and transferred to a tube of peptone bouillon, which was then incubated at 37° C. After 48 hours, $\frac{1}{2}$ c.c. of this incubated bouillon was transferred to a fresh tube of bouillon and incubated for 48 hours longer, after which agar plates were made with the contents of all the bouillon tubes. All the culture tubes and plates were examined macroscopically and microscopically repeatedly for the purpose of finding whether growth had taken place in any of them. Control tests showed that all the cultures tested were capable of active growth.

A second set of test cultures was prepared after the original cultures had been exposed to a temperature of 98.5° for two minutes, and a third set of tests was started after the exposure at 98.5° had lasted 20 minutes. The results of these tests are given in the following table:—

TABLE XIII.
First set of experiments on thermo-lethal point of some milk bacteria.

KINDS OF BACTERIA TESTED.	REACTION OF GROWTH IN LITMUS LACTOSE AGAR.	SOURCES OF THE BACTERIA TESTED, AND REFERENCES TO SAMPLES.	CULTURES NOT HEATED. CONTROL.		CULTURES HEATED IN WATER BATH.					
					80° C. for 20'.		98.5° C. for 2'.		98.5° C. for 20'.	
					Sub-culture after		Sub-culture after		Sub-culture after	
			2 days 37° C.	5 days 20° C.	2 days 37° C.	5 days 20° C.	2 days 37° C.	5 days 20° C.	2 days 37° C.	5 days 20° C.
Streptococcus, short	Acid	Whole fresh Milk A 2 ...	†	††	0	0	0	0	0	0
"	"	Dry Milk R 2 ...	†	††	0	0	0	0	0	0
Staphylococcus ...	"	" E 2 ...	†	††	0	0	0	0	0	0
"	"	" E 3 ...	†	††	0	0	0	0	0	0
Sarcina ...	Slightly acid	" R 3 ...	†	††	0	0	0	0	0	0
Thick sporing Bacillus ...	Neutral	" D f ...	††	†††	††	†††	††	†††	†††	†††
Streptothrix white	"	" D a ...	?	†	0	0	0	0	0	0
" brown	"	Pasteurised Milk Pa ...	?	†	0	0	0	0	0	0

Except with regard to the cultures of the white and of the brown streptothrix, which were of very slow growth, the results of this first set of experiments appeared to be conclusive. I thought it desirable, however, to repeat the experiments, using in every case larger quantities of cultures mixed with milk. In the case of the two kinds of streptothrix, three months old typical cultures on potato were used.

In the second set of tests which were carried out more than one year after the first, each exposure was made in a narrow tube with thin walls, plunged in a water bath at constant temperature. The contents of the tubes reached the temperature of the water baths in less than half a minute. At the end of the period of exposure *the whole of the contents* ($\frac{1}{2}$ c.c.) of each tube was divided immediately between a tube of peptone bouillon and an agar plate.

In one group of experiments the bacterial emulsions of young cultures were exposed to a temperature of 85° for one minute, five minutes and 20 minutes respectively. In another group the bacterial emulsions of old cultures (97 days)* were kept respectively at 80° C. for 20 minutes, at 88° to 90° for five minutes, and at 99.5° for two minutes. The results of these two groups of experiments are summarised in Table XIV.

* I had previously ascertained that cultures of all the bacteria used were still capable of active growth after being kept 148 days

TABLE XIV.—Second set of experiments on the thermo-lethal point of some milk bacteria.

KINDS OF BACTERIA TESTED.	REACTION ON LITMUS LACTOSE AGAR.	SOURCES OF THE BACTERIA TESTED AND REFERENCES TO THE SAMPLES.	MIXTURE KEPT AT 85° C. FOR 1'.		MIXTURE KEPT AT 85° C. FOR 5'.		MIXTURE KEPT AT 85° C. FOR 20'.	
			MIXTURE NOT HEATED.		MIXTURE KEPT AT 85° C. FOR 1'.		MIXTURE KEPT AT 85° C. FOR 5'.	
			Control 1 loopful of mixture in bouillon. agar plate. 1 week.	1 c.c. of mixture in bouillon. 1 week.	1 c.c. of mixture on agar. 1 week.	1 c.c. of mixture in bouillon. 1 week.	1 c.c. of mixture on agar. 1 week.	1 c.c. of mixture on agar. 1 week.
1st group, 48 hours sub-culture in bouillon incubated at 37° C. (except streptothrix).	Streptococcus, short	Whole fresh Milk A 2	++	o	o	o	o	o
	" long	Dry Milk from hot chamber R 4	++	o	o	o	o	o
	Staphylococcus, small	Dry Milk sieved E 3	+++	o	o	o	o	o
	Coccus (sarcina?), large	Dry Milk from hot chamber R 3	++	o	o	o	o	o
	Sarcina, yellow	Dry Milk sieved E 4	+++	o	o	o	o	o
White streptothrix* (old culture)		Whole fresh Milk A j	++	o?	o?	o?	o?	o?
			++	o?	o?	o?	o?	o?
2nd group, 97 days old cultures.			80° C. FOR 20'.		88 TO 90° FOR 5'.		99.5° FOR 2'.	
White streptothrix*	Neutral	Whole fresh Milk A g	++	o?	o?	o?	o?	o?
Brown streptothrix*	"	Pasteurised Milk P a	+	o?	o?	o?	o?	o?
Sarcina, pink	Alkaline	Dry Milk from hot chamber R 5	++	o	o	o	o	o
Large Bacillus sporing (B. mesentericus niger)	Neutral or alkaline	Dry Milk from hot cylinder B 1	+++	+++	+++	+++	+++	+++
Large Bacillus sporing (B. mesentericus ruber).	"	Dry powdered Milk sieved D f.	+++	+++	+++	+++	+++	+++

* All the doubtful cultures of Streptothrichae were examined again six weeks after inoculation and no evidence of growth could be discovered in any.

These experiments show that the only bacteria, among those isolated from the preserved milk ready for sale, which offer a marked resistance to the amount of heat to which they were exposed during the treatment of the milk are sporing bacilli, most of which belong to types loosely grouped together under the name *B. mesentericus*.

Other sporing bacilli, as for instance, the *B. subtilis*, the *B. enteritidis sporogenes*, &c., would undoubtedly behave in the same way.

The results obtained with the streptothrichae were not quite clear. These organisms were undoubtedly much affected, if not actually killed, by short exposures to moderate heat, but the slowness of their growth, and the fact that filaments having all the appearances and staining properties of growing filament were found in culture media in which no clear colonies (such as those present in the control cultures) could be discovered after long periods suggests the necessity of further tests. The comparatively large number of colonies of streptothrichae obtainable from some of the samples of powdered milk, suggests that even if the presence of a few of these organisms can be attributed to their resistance to the heat to which they are submitted during the preparation of dry and condensed milk, there must be in addition recontamination. It is probable that the dust of a place infected with streptothrichae is, when disturbed frequently, loaded with their gonidia, which infect operators, vessels, water, air, etc.

With regard to the streptococci, staphylococci and sarcinae, which were so abundant in the finished article, it is clear that they are not capable of resisting the heat to which they are submitted during the process of manufacture.

These results therefore confirm the conclusion which the direct examination of the milk at various stages of treatment had indicated, and it is certain that the presence of most of the bacteria found in preserved milk as sold is due to contact with various contaminated objects or persons and to exposure to dust. The fact that the fresh milk before treatment contains most of the bacteria that are found in the prepared milk shows that both the fresh and the prepared milk derive most of these bacteria from the same sources, which are water, soil and dirt acting directly or through the intermediation of air currents, vessels, persons, &c.

The previous remarks apply specially to saprophytic bacteria. The tubercle bacillus will now be considered.

B. Thermo-lethal point of tubercle bacilli.

In connection with the experiments conducted at Factory V, the heating of milk from 12° to 78° in 29 minutes (according to the method of so-called "pasteurisation" used at the condensery) caused a considerable reduction in the total number of bacteria, and more specially in the number of bacteria liquefying gelatine. But a number of tubercle bacilli survived, and were capable of producing tuberculosis by inoculation in one out of two guinea pigs inoculated (the second guinea pig died prematurely). One out of two guinea pigs inoculated with the same milk, heated for ten minutes more, during which the temperature was raised to 92°, became also tuberculous.

In the two guinea pigs infected by this heated milk the tuberculous lesions had an infective character and were clearly due to the presence of *virulent living* tubercle bacilli in the milk tested.

There was a marked retardation in the appearance of the tuberculous lesions. In fact the *disease remained latent* for about three weeks, but in one animal kept under observation for two months and 18 days these lesions had become so extensive that very few organs had remained free from tuberculosis.

These results are in perfect agreement with those I obtained in 1899, when conducting experiments on sterilization of tuberculous milk and cream. I have recorded some of these experiments in a paper on the "Stamping out of Bovine Tuberculosis," which I read in 1901 before the British Congress on Tuberculosis (Transactions of the British Congress on Tuberculosis, Vol. II., p. 235, London, 1902). The following table is taken from that paper:—

TABLE XV.

General results of experiments on the sterilisation of tuberculous cream.

	Temperature.		Duration of Exposure after the Temperature indicated had been reached.	Result as indicated by comparison with control.
	Centigrade.	Fahrenheit.		
1st set of experiments (1899)	74° C.	165°	30 minutes	Tuberculosis slight and delayed, but distinct in 3 weeks.
" " "	76°	168.8°	10 "	Tuberculosis slight and delayed, but distinct in 3 weeks.
" " "	82.5°	180.5°	5 "	Tuberculosis well marked. Nearly normal.
2nd set of experiments (1899)	85°	185°	15 "	Tuberculosis considerably delayed (2 months).
" " "	70°	158°	45 "	Tuberculosis delayed, but well marked in 3 months.
" " "	65°	149°	360 "	No tuberculosis in 3 months.*
" " "	65°	149°	180 "	Slight tuberculosis in 1 month.
" " "	64°	147.2°	60 "	No tuberculosis after 1 month.*
2nd set of experiments (1899)—Control animals inoculated with the same amount of infected cream as in other experiments	Not heated			Well marked tuberculosis in 3 weeks.

* Lesions may have been present in these experiments, but so slight as to escape detection; it would have been necessary to keep the animal longer to obtain conclusive results.

These experiments, in some of which Dr. Coutts assisted me, were conducted with considerable care, and convinced me that cream infected with human tubercle bacilli was not invariably sterilized when exposed to a temperature of 85° C. even when the exposure lasted 15 minutes.

I subsequently obtained similar results with milk containing tubercle bacilli of bovine origin. The presence of living tubercle bacilli in the treated cream or milk was proved by inoculation experiments, the procedure which I adopted in my experiments made it easy to distinguish lesions produced by living, from those produced by dead bacilli. Lesions produced by living bacilli are infective, *i.e.*, they are not limited to the seat of inoculation and neighbouring tissues, but occur also in organs situated at a distance from the seat of inoculation. In several cases I satisfied myself that the bacilli contained in these organs were living and active by further inoculations and cultivations.

When intraperitoneal inoculations are used, as has been done by the majority of observers, the lesions produced by dead bacilli may easily, as Rosenau justly says, be mistaken for lesions produced by living bacilli. This, however, is only a reason for discarding the use of peritoneal inoculation. I am therefore obliged to differ from the opinion expressed by several observers, and recently reaffirmed by Rosenau in his interesting paper on the "Viability of the Tubercle Bacillus" (Bulletin, Nos. 42 and 57, Hygienic Laboratory, Public Health and Marine Hospital Service of the United States, 1909), from which the following table is taken:—

TABLE XVI.

Showing the thermal death point of the tubercle bacillus as found by various investigators.

INVESTIGATOR.	KILLED AT—	NOT KILLED AT—
Martin 1882	—	80° C.
May, 1883	By cooking	
Sormani, 1884	Boiling, 5 minutes	90° for 10 minutes.
Schill and Fisher, 1884...	—	100°.
Voelsch, 1887	—	100° boiling twice.
Yersin, 1888	{ 60°, 10 minutes (— spores) ...	
	{ 60°, 10 minutes (+ spores) ...	
Bitter, 1890	{ 68°, 20 minutes	
	{ 70°, 5 minutes (enfeebles) ...	
	{ 60°, 5 minutes (sometimes	
Bang, 1891	enfeebles)	
	{ 80°, (sometimes kills) ...	
	{ 85°, (always kills)	
Bonhoff, 1892	{ 60°, 20 minutes	50°, 60 minutes.
Grancher and Ledoux-	{ 60°, 5 minutes (attenuates) ...	
Lebard, 1892	{ 70°, 1 minute (kills)	
Forster, 1892	{ 60°, 6 hours	{ 55°, 3 hours.
	{ 95°, momentary	{ 60°, 5 minutes.
		{ 80°, momentary.
De Man, 1893	{ 55°, 4 hours	
	{ 60°, 1 hour	{ 60°, 45 minutes.
Schroeder, 1894	{ 60°, 15 minutes	

INVESTIGATOR.	KILLED AT—	NOT KILLED AT—
Woodhead, 1895	$\left\{ \begin{array}{l} 50^{\circ}, 15 \text{ hours} \dots \dots \dots \\ 60^{\circ}, 8 \text{ hours} \dots \dots \dots \\ 60^{\circ}, 45 \text{ minutes} \dots \dots \dots \\ 70^{\circ}, 45 \text{ minutes} \dots \dots \dots \\ 70^{\circ}, 2\frac{1}{2} \text{ minutes} \dots \dots \dots \end{array} \right.$	$\left. \begin{array}{l} 90^{\circ} \text{ (results contradictory).} \\ 60^{\circ}, 10 \text{ minutes.} \end{array} \right\}$
Marshall, 1899 ...	68°, 20 minutes	
Th. Smith, 1899	60°, 15 to 20 minutes	
Morgenroth, 1900	$\left\{ \begin{array}{l} 55^{\circ}, 3 \text{ hours} \dots \dots \dots \\ 70^{\circ}, 10 \text{ minutes} \dots \dots \dots \\ 100^{\circ}, \text{momentary} \dots \dots \dots \end{array} \right.$	
Kobrak, 1900 ...	50°, 4 hours	
Beck, 1900 ...	100°, 3 hours	$\left\{ \begin{array}{l} 100^{\circ}. \\ 80^{\circ}, 30 \text{ minutes.} \\ 85^{\circ}, 6 \text{ minutes.} \end{array} \right.$
Galtier, 1900 ...	—	
Russell and Hastings, 1900	60°, 20 minutes	80°, 5 seconds.
Herr, 1901 ...	65°, 15 minutes	
Hesse, 1901 ...	60°, 20 minutes	
Levy and Burns, 1901	65°, 15 minutes	70°, 15 minutes.
Barthel and Stenström, 1901	—	60°, 15 minutes.
Bang, 1902 ...	—	
Tjaden, 1903 ...	85°, 1 to 2 minutes	60°, 20 minutes.
Rullmann, 1903...	65°, 30 minutes	80°, 1 minute (coagulated).
Barthel and Stenstrom, 1904	80°, 1 minute (uncoagulated)	
Russell and Hastings, 1904	71°, 1 minute...	
Zelenski, 1906 ...	—	76°, 20 minutes.
Rosenau, 1907 ...	60°, 20 minutes	

Rosenau states that “the tubercle bacillus in milk loses its infective properties for guinea pigs when heated to 60° C. and maintained at that temperature for 20 minutes, or to 65° C. for a much shorter time.”

Rosenau is under the impression that experimenters holding different views have been misled by the lesions produced by dead tubercle bacilli, but although this may be true of some of the experiments quoted by him, it does not apply to several other experiments, including my own (which have been overlooked by him), in which the survival of bacilli in milk heated to temperatures ranging from 64° C. to 92° C. for lengths of time exceeding those mentioned by him, was proved beyond doubt, by the adoption of the method of inoculation which I recommended in 1893 for the very purpose of detecting the presence of *living tubercle bacilli* in various products.

C. Effects of the addition of sugar to condensed milk upon the tubercle bacillus.

After the addition of sugar subsequently to heating to 92° C., and further heating in vacuo, for about one hour to a temperature not exceeding 50° C., the milk had lost the power of producing tuberculosis. Six guinea pigs, inoculated with the sediment of comparatively large quantities of condensed milk prepared at Factory V with tuberculous milk, remained free from any tuberculous lesion for 40 and 51 days respectively in two cases, and 78 days in the four other cases. These results are also in agreement

with those obtained in my laboratory between the years 1900 and 1908 with condensed milk of various makes. During that period 33 guinea pigs were inoculated with various kinds of condensed milk, and none of these animals contracted tuberculosis. Eight of these animals had been inoculated with four different samples of condensed milk, prepared according to the method used at Factory V.

Several of these samples produced more or less localised inflammation, but none of the lesions was tuberculous, the bacteria present in the parts affected were generally cocci, in one case, however, a bacillus allied to the *Bacillus enteritidis* (Gaertner) was found.

In the experiments recorded in this report the addition of sugar to the milk seemed to produce some alteration in the tubercle bacilli, for they were more difficult to find in the condensed than in the original milk.

The morphological characters of the bacilli found in the condensed milk were altered, the bacilli were smaller and more granular than those present in the original milk before condensation.

D. *Temperature reached by the milk dried over revolving cylinders. (Method B.)*

The presence of living tubercle bacilli in milk dried over cylinders heated to 138° C. to 140° C. is by no means so extraordinary as would appear at first sight.

When the milk passes down through the narrow (1 to 2 mm.) space between the two cylinders, the tension of the steam produced on the surface of the thin layer of milk keeps the milk at a small distance from the surface of the cylinder.

The temperature of the surface of the cylinder is less than that which, according to Boutigny, should be necessary to cause water to pass into the spheroidal state. It is however, probable that the separation of the layer of milk from the cylinder is attributable to the production of a state similar to the spheroidal state. Boutigny has also shown that the temperature of a fluid in the spheroidal state is below the boiling point of that fluid. For water the temperature is stated to be 95.5°. For milk the temperature would probably not be much higher.

The high temperature of the cylinder does not therefore affect the bacilli contained in the milk passing over the surface as much as if the milk had been simply brought to the boiling point by being heated in the usual way in an ordinary vessel.

I have ascertained that the temperature of part of the milk nearly reaches the boiling point during the short time it remains in the gutter formed between the upper halves of the two cylinders (see Diagram 4), but, as during the first experiment, 10 gallons of milk passed in eight minutes through that space, and during the second experiment 17 gallons passed in 13½ minutes, a great part of the flowing milk must have remained at a temperature below 100° C., and in all probability the maximum temperature reached by every part of the milk treated did not exceed

96° C., the duration of the exposure to that temperature probably never exceeded 3.3 seconds.*

The process as conducted at present cannot therefore be expected to yield a sterile product.

VIII. FEEDING EXPERIMENTS.

For the purpose of these experiments young rabbits, ranging in weight from 300 grammes to 550 grammes, were kept under observation for two weeks. All those which, under normal conditions, gained weight and appeared healthy, were separated and caged in pairs, after which each pair of animals was given daily for eleven days 60 grammes of crushed oats and 60 c.c. of water. At the end of that period all the animals that had gained weight were retained. Each pair then received daily 60 grammes of crushed oats and 60 c.c. of the milk to be tested.† After a further interval of six days the quantity of oats given to each animal was increased to 40 grammes, and four days later, in addition to the 30 c.c. of milk given in the morning, 30 c.c. of water was given in the afternoon. Each experiment, including the first eleven days of preliminary trial, lasted from March 10th to May 18th (except in the case of some of the rabbits which died prematurely). In order to ascertain the state of health of the rabbits, note was taken of the way in which they took their food, of any evidence of diarrhoea, and of their weight. Each animal was weighed daily at 9 a.m. The food was given immediately afterwards, it was almost invariably all eaten within two hours. Water, as previously stated, was given after a time every afternoon because the rabbits appeared to need it.

The experiments were arranged as follows:—

2,158 and 2,159 (four rabbits). *Dry milk*, made up with sterile water. During the first eight days the proportion was 1 part of dry milk to 4 parts of water, after which the proportion was 1 part of milk to 6 parts of water.

2,160 and 2,161 (four rabbits). *Condensed milk* made up in the proportion of 1 part of milk to 3 parts of water.

* The extreme speeds at which the cylinders are intended to move are 6 and 14 revolutions per minute. The pellicle is separated after the cylinder has made two-thirds of a revolution. The time of contact is therefore intended not to exceed 6.6 seconds and may be reduced to 2.8 seconds. In our experiments on the treatment of whole milk the speed was about 14 revolutions per minute. For the treatment of separated milk the speed was reduced to about 12 revolutions, which corresponds to a contact of 3.3 seconds' duration.

† It will be noticed that I did not attempt to feed the rabbits entirely on milk. I had several reasons for using a mixed diet. (1) The rabbits had been on mixed diet for some time (I could not obtain in time a sufficient number of younger rabbits); (2) I thought it desirable that the milk should not remain for more than one or two hours in the cages, and that the whole dose given should be taken at once; (3) I desired to continue the administration of milk for at least one month, and the quantity of some of the treated milk at my disposal would not have allowed me to give larger daily doses. The experiments were made specially for the purpose of testing the infectivity of the milk, the question of nutrition was necessarily of secondary importance. No attempt was therefore made to construct a rational diet. This, however, does not diminish the value of the experiments for comparison purposes.

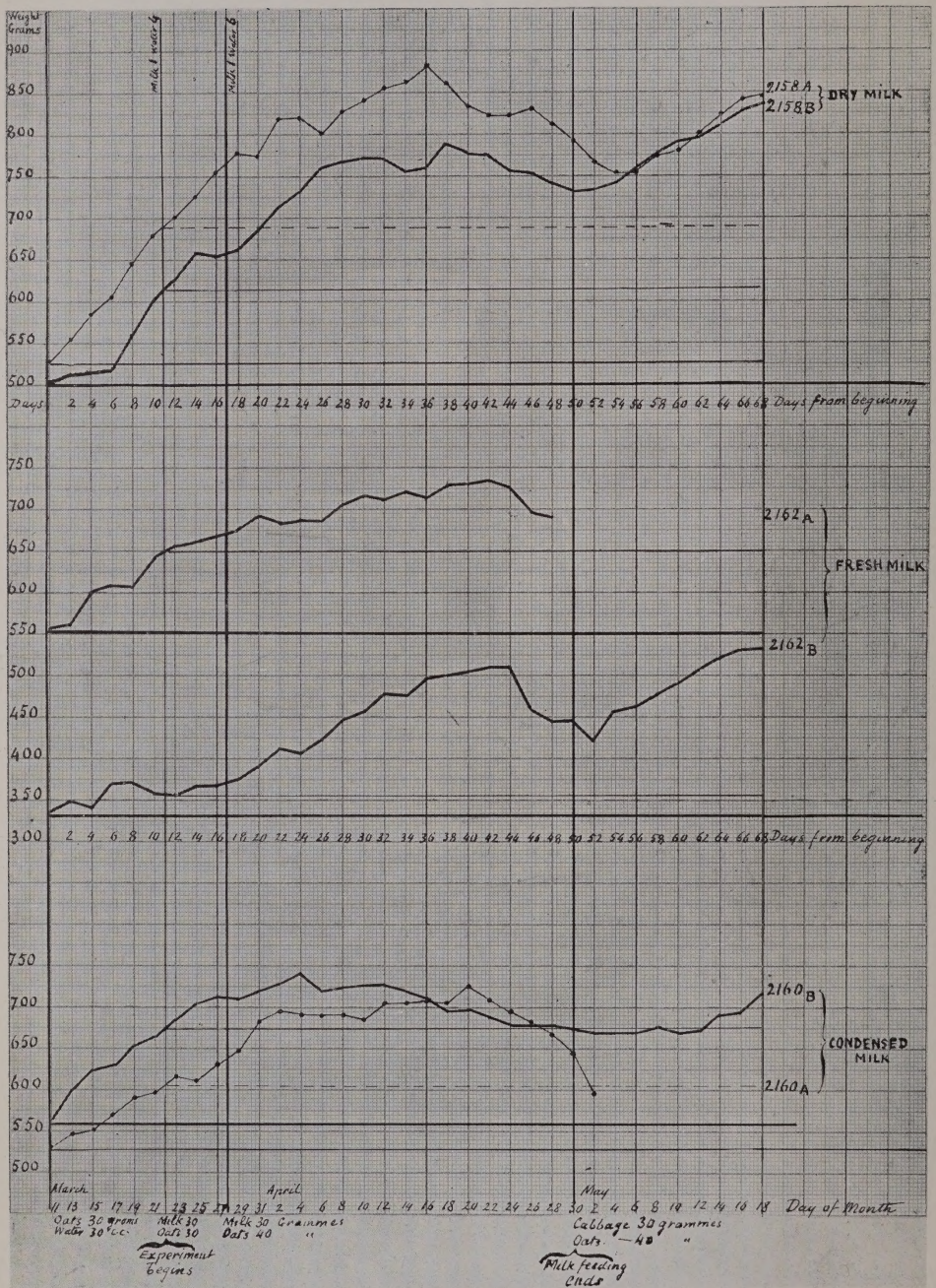


DIAGRAM 7. WEIGHT CURVES OF RABBITS FED ON DRY MILK, SWEETENED CONDENSED MILK AND FRESH MILK.

(For explanation see text.)

2,162 and 2,163 (four rabbits). *Fresh cow's milk.**

Each kind of milk was divided into two portions which were treated in the following ways—one portion used for feeding two animals was prepared or collected in the evening and kept in the refrigerator till the next morning; the other portion was incubated during the night at 22° C. for the first eleven days and afterwards at 30° C., and used next morning for feeding a second pair of rabbits. The milk was therefore either refrigerated or incubated for about 16 hours. All the kinds of milk tested, and all the animals experimented upon were treated strictly in the same way.

The refrigerated milk diet seemed to agree with all the rabbits for about one month, after which the animals generally began to lose weight, but on replacing 30 c.c. of milk by 30 grammes of cabbage, they gradually regained the lost weight.

The effect of feeding with various kinds of milk is best shown by the curves of weights (see Diagram 7), but it can generally be stated that the rabbits fed with the *refrigerated* reconstituted dried milk increased more rapidly in weight than those fed on *refrigerated* fresh or condensed milk, and that when they were killed 57 days after the beginning of the experiments all their organs were healthy. (It may at once be stated here that all the other animals fed on refrigerated fresh or condensed milk were also found quite healthy when killed.) Two of the rabbits, viz., one fed on condensed and one on fresh refrigerated milk, died before the end of the experiment. The rabbits fed on *incubated* reconstituted dry milk, condensed milk, and fresh milk *all died* before the end of the experiment with the exception of one rabbit fed on fresh incubated milk. Two of these animals, one of them fed on dry incubated milk, and the other on fresh incubated milk had suffered from acute gastro-enteritis before death, but there was no clear evidence that the milk had been the source of infection, for there was another possible cause of disease.

None of the four rabbits fed on the dry tuberculous milk nor of the four rabbits fed on condensed tuberculous milk showed any evidence of tuberculous infection, although 1,200 c.c.† of each kind of milk had been administered to each animal in the course of 40 days. These experiments are not numerous enough to be absolutely conclusive, but they are sufficient to show that the results of the inoculation experiments may perhaps give an exaggerated idea of the possible dangers connected with incomplete sterilization.

On comparing the curves of weights, it is clear that the animals fed on dry milk diluted seven times, gained weight more rapidly

* Fresh cow's milk was received at the laboratory less than two hours after milking. Each day one cow was milked direct into a sterilized vessel supplied for the purpose. During the experiment milk from 27 cows was used. In this way absolutely fresh milk of average quality was used for comparison with the prepared milk.

† i.e., more than twice the weight of each animal.

than those fed on condensed milk diluted four times, or on undiluted normal milk.

The superiority of dry milk over fresh milk observed in this set of experiments may be purely accidental, but with regard to the differences between dry milk and condensed milk, the explanation is obvious, for even though the condensed milk was diluted only four times, the reconstituted condensed milk was poorer in proteid and fatty matter than the reconstituted dry milk, this being due to the fact that more than half of the solid constituents of condensed milk consists of cane sugar.

The advantages of dry milk, from the hygienic as well as from the commercial point of view, are very great. It is therefore regrettable that the process of drying should not always yield an article entirely free from pathogenic bacteria. It is, however, clear that, if clean milk obtained from healthy cows were dried in a factory where suitable precautions were taken to prevent *recontamination*, dry milk almost free from bacteria could be produced.

IX. GENERAL SUMMARY.

1. The total number of bacteria present in mixed cows' milk, such as is usually supplied to town consumers,* has been found to be considerably reduced by treatment according to each of the three methods investigated. The reduction was greatest in the case of Method A (manufacture of sweetened condensed milk), and least in the case of Method C (drying of milk sprayed in a current of hot air). Method B (drying of milk over heated revolving cylinders) occupied an intermediate place.

2. In each of the three methods of treatment there was a stage at which the reduction in the total number of bacteria was much greater than that observed in the finished article ready for sale.

3. The increase in the number of bacteria observed during the final stages is due to *recontamination*.† By recontamination I mean the results of the exposure of a product partly or completely sterilized, to sources of infection by which some of the bacteria removed by sterilization are reintroduced.

4. The reduction in the total number of bacteria was almost entirely due to the death of Streptococci, Staphylococci, Sarcinae, Bacilli of the B. Coli type, Streptothrichae, yeasts, &c.

5. At none of the stages of preparation was the milk ever found completely sterile. The amount of heat to which the milk was submitted was insufficient to bring about the death of several saprophytic and of some pathogenic bacteria.

6. Among the saprophytic bacteria, which were invariably found to resist pasteurisation, those most commonly detected were sporing bacilli of the types included under the term *Bacillus*

* That is, milk in which extraneous bacteria have generally had the opportunity of multiplying to various extent.

† In fixing *bacterial standards* for preserved milk this fact should be kept in mind, for it is clear that by the exercise of proper care *recontamination* might be almost entirely prevented. If this were done the total number of aerobic bacteria, cultivable by the methods indicated in this report should seldom exceed 100 per gramme of preserved milk.

mesentericus. Some Streptothrichae appeared in some cases to have survived, but the evidence on that point was not conclusive.

7. Of the pathogenic bacteria, the tubercle bacillus was the only one the fate of which was investigated. Some living tubercle bacilli of bovine origin were found to have survived treatment according to Method B.* It may be safely assumed that Method C, which yields a product giving a higher total bacterial count than Method B, has even less effect upon tubercle bacilli. The same bacilli resisted the process of pasteurisation which forms part of Method A.

8. The tubercle bacilli which had survived pasteurisation in Method A and drying by heat in Method B, were still capable of producing progressive tuberculosis in guinea pigs inoculated subcutaneously with milk containing these bacilli, but the course of the disease produced by these bacilli was very much slower than that of the disease produced in guinea pigs inoculated with the same amount of untreated tuberculous milk. The tuberculosis produced by the heated bacilli was *latent* or *occult* for some four weeks.

Young rabbits fed with milk containing these modified bacilli did not contract tuberculosis.

SHERIDAN DELÉPINE.

A. W. J. MACFADDEN,
Chief Inspector of Foods,
December, 1914.

* I am aware that the above conclusions, except those relating to feeding, do not agree with those arrived at by other observers in the United States and in Germany. Thus, Dr. W. Hoffmann, of the Kaiser-Wilhelm Akademie, is reported to have stated that he had proved by experiments that the *Bacilli of Bovine Tuberculosis which may be present in liquid milk are killed by one of the methods of drying milk tested by us*. Whether the difference between his results and mine is due to a difference in the original number of bacilli, to a difference in the characters of the milk treated, or to the way in which the process was carried out, I cannot say, but the precautions which I have taken to avoid accidental contaminations entitle me to say positively that under the conditions which I have detailed in this report sterilisation was not complete.



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